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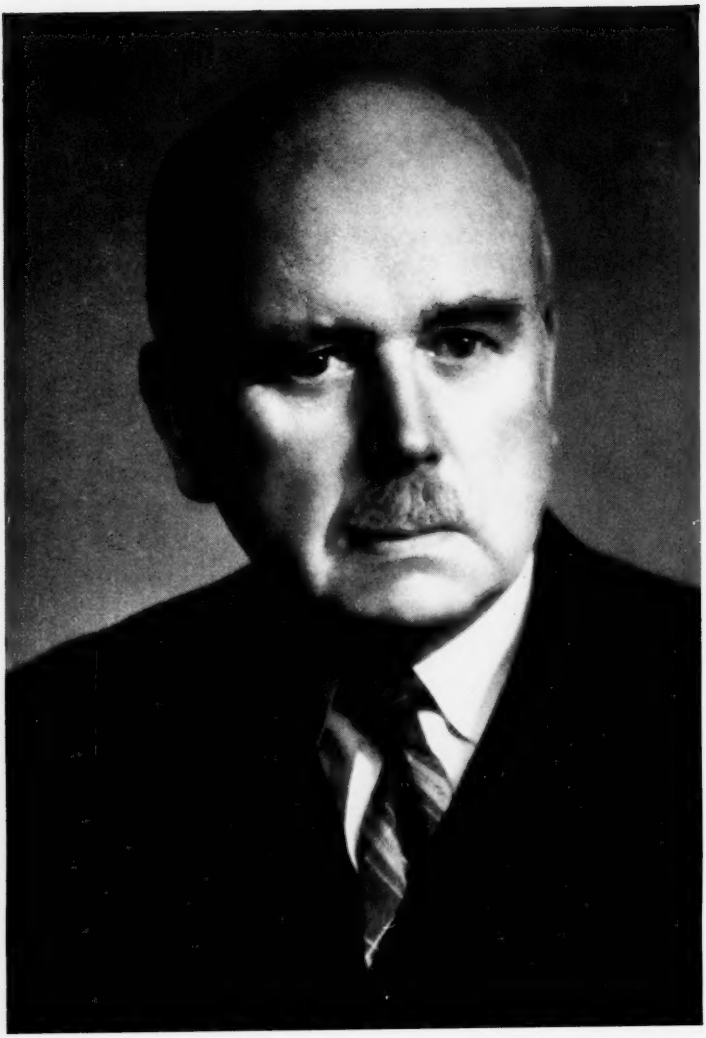

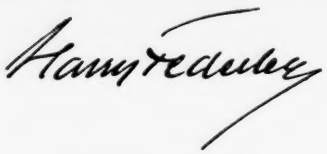

HARRY FEDERLEY

22. 3. 1879 — 13. 11. 1951

In memoriam

PACHYTENE ANALYSIS OF A DEFICIENT ACCESSORY CHROMOSOME IN RYE

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IN rye, *Secale cereale*, the normal chromosome number is $2n = 14$, but in various rye populations from widely different parts of the distribution area of the species plants have been found which contain supernumerary chromosomes of a quite special kind. As a rule, the deviating plants in question contain 2 so-called »standard fragments» in addition to the 14 normal chromosomes. The standard fragment is about half as big as an ordinary rye chromosome and is characterized by having a subterminal centromere. At mitosis the short arm of this chromosome is headlike, the long arm being about five times as long as the short arm (*cf.* MÜNTZING, 1944, Figs. 6—7).

The standard fragment behaves normally in all somatic mitoses, but at the first division in the pollen grain it undergoes a directed non-disjunction, both chromatids passing to the generative nucleus (MÜNTZING, 1946). The same phenomenon occurs on the female side during the first division in the embryo-sac (HÅKANSSON, 1948). For this reason plants with $2n = 14 + 2$ s. f., when intercrossed, may give an offspring in which most of the plants have $2n = 14 + 4$ s. f. (*cf.* MÜNTZING, 1949, Table 1). Such plants, in their turn, tend to give offspring with still higher numbers of standard fragments. However, the increase caused by non-disjunction is counterbalanced by the deleterious genetic effects of the standard fragments, plants with such accessory chromosomes having a clearly reduced fertility and a slightly reduced vigour in comparison with plants lacking such chromosomes (MÜNTZING, 1943, 1949).

In cultures of rye with accessory chromosomes (»B-chromosomes») new types of accessory chromosomes have appeared. Thus, a so-called large iso-fragment has been obtained, which has arisen by misdivision of the centromere. As definitely demonstrated by a detailed pachytene analysis (MÜNTZING and LIMA-DE-FARIA, 1949), the arms of this iso-chromosome are both identical with the long arm of the standard fragment. The corresponding small iso-chromosome, representing twice the short arm of the standard fragment, has probably been obtained as

well, but in this case the pachytene analysis has not yet been carried out.

An interesting difference between the large and small iso-fragment is that the former has perfectly well retained the ability of undergoing non-disjunction, whereas the small iso-fragment is incapable of performing this peculiar phenomenon. The same is true of a third derivative of the standard fragment, the so-called deleted fragment (MÜNTZING, 1948). Judging from the appearance of this chromosome in somatic metaphases the short arm is intact, the loss being confined to about half of the long arm.

The loss of the ability of undergoing non-disjunction, characteristic of the small iso-fragment as well as of the deleted fragment, seemed to fit in very well with the mechanism of non-disjunction observed in the standard fragment as well as in the large iso-fragment. The configurations observed at anaphase of the first pollen mitosis strongly indicated that in the first place a special segment in the long arm, not far from the centromere, was responsible for the process of non-disjunction. This segment is absent in the small iso-fragment and was supposed to have been also lost in the deleted fragment (MÜNTZING, 1948).

Now, to procure more information on this point it is necessary to make a detailed pachytene analysis of all chromosome types involved. As already mentioned, this has been done with regard to the standard fragment and the large iso-fragment. In the cases of the small iso-fragment and the deleted fragment a serious obstacle arises due to the fact that, owing to lack of non-disjunction, it is very difficult to get plants having more than one chromosome of these kinds. Practically all plants with such chromosomes have them in a single dose, and in such plants it is not easy to study the detailed structure of the fragment chromosome at pachytene, owing to the fact that the chromosome in question has a tendency to pair with itself.

Some data concerning the transmission of the deleted fragment were previously given (MÜNTZING, 1948, pp. 437—438). In plants with $2n = 14 + 1$ deleted fragment a high degree of meiotic elimination occurs, and only about 7,5 per cent of the gametes (male as well as female) were found to transmit the fragment. Consequently, in offspring derived from intercrosses between plants with one deleted fragment the ratio between plants with 0, 1 and 2 fragments should be $85{,}56 : 13{,}88 : 0{,}56$. The proportion actually observed ($82{,}3 : 16{,}1 : 1{,}6$) is in good accordance with the expected ratio.

These data were based on 62 individuals including a single plant

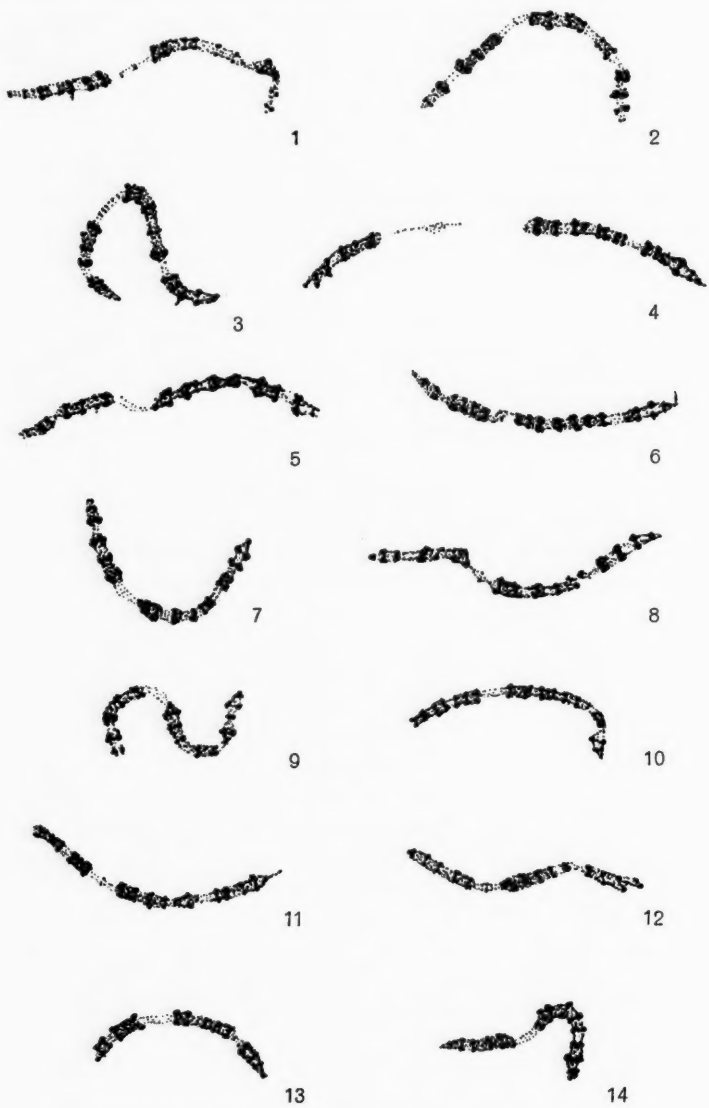
with 2 deleted fragments. This plant was used for studies of first metaphase and later meiotic stages but not for pachytene, the technique necessary for such work (LIMA-DE-FARIA, 1948) not being available at that time.

Since then new attempts have been made to obtain plants with 2 deleted fragments from intercrosses of plants having one accessory chromosome of this kind. The following results were obtained (Table 1). As is evident from the table, none of the 98 plants raised in 1949 had two deleted fragments, the 12 plants carrying such chromosomes having them in a single dose. However, in 1950 2 plants among a total of 182 had 2 deleted fragments, and in 1951 one such plant was obtained among 178 individuals examined. Pachytene fixations of these plants could be obtained. For this work vegetative propagation of the plants proved to be quite useful.

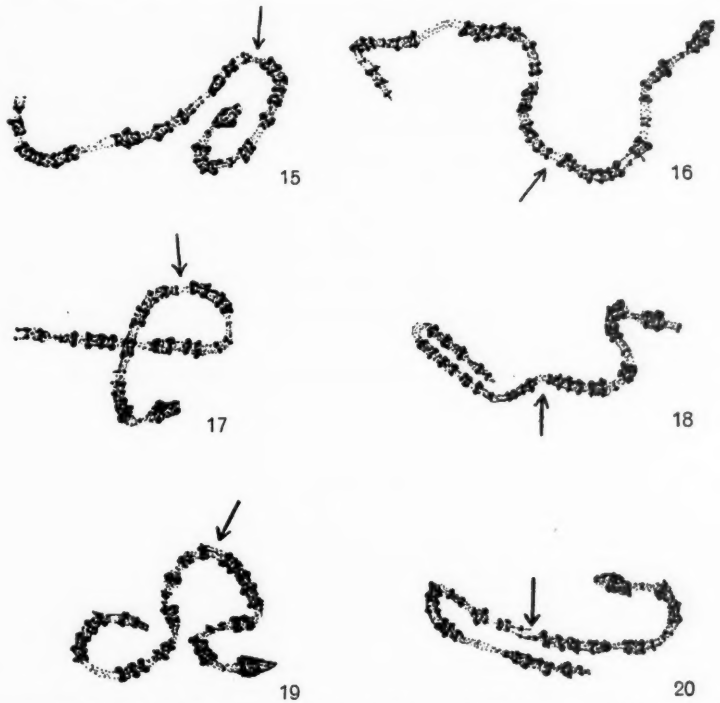
Before describing the cytological details it should be pointed out that there is a significant heterogeneity in the frequencies of plants with deleted fragments, the frequency being higher in 1950 than in the other

TABLE 1. *Progenies of intercrossed plants having* $2n = 14 + 1$ *deleted fragment.*

Year and progeny	Number of deleted fragments 0	1	2	n	Per cent plants with d. f : s
1949—17	14	3			
—18	26	3			
—19	26	3			
—20	20	3			
1949, total	86	12	0	98	12.24
1950—131	25	9	2		
—132	33	6			
—133	20	9			
—134	30	8			
—135	31	9			
1950, total	139	41	2	182	23.63
1951—517	27	3			
—518	24	3			
—519	17	6			
—520	23	3			
—521	24	1	1		
—522	24	1			
—523	21	0			
1951, total	160	17	1	178	10,11



Figs. 1—14. Paired deficiency fragments at pachytene. — × 2500.



Figs. 15—20. Paired standard fragments at pachytene. Limit between regions 1 and 2 in the long arm indicated by an arrow. — × 2500.

two years. According to Table 1 the percentages of plants with deleted fragments in 1949, 1950 and 1951, were 12,24, 23,63 and 10,11, respectively. A χ^2 test of heterogeneity of the ratios 86 : 12; 139 : 43 and 160 : 18 gave a χ^2 value of 13,54. This corresponds to a P value intermediate between 0,01 and 0,001. As the different families within years evidently behave very similarly, it must be concluded that differences between the environmental conditions in the different years must have influenced the degree of meiotic elimination of the single deleted fragment in the mother plants or the relative capability of pollen grains and embryosacs with the fragment to function at fertilization.

The appearance of the deleted fragment at pachytene is shown in Figs. 1—14. For comparison six standard fragments at the same stage and of the same variety (Östgöta Gråråg) are represented by Figs. 15—

20. The most conspicuous difference between the two chromosome types is that the deleted fragment lacks the characteristic terminal knob formation present in the long arm of the standard fragment, and that the long arm in the deleted fragment is only about half as long as in the standard fragment. The short arm, on the contrary, has about the same appearance in the two chromosome types.

A detailed chromomere analysis revealed that the »deleted» fragment had suffered a *terminal deficiency*, the entire terminal half of the long arm being lost. The point of breakage could be clearly localized and is indicated by an arrow in Figs. 15—20. At this point the long arm shows a particularly weakly stained fibrilla pair. Several other points

Fig. 21. Comparison between standard fragment (above) and deficiency fragment (below). Region 2 of the long arm of the standard fragment is absent in the deficiency fragment. — × 2500.

in both arms of the standard fragment have a similar appearance, the most conspicuous one being the »neck» region close to the knob. Thus, the chromomeres are not evenly distributed but assembled in groups of various sizes separated by particularly weakly stained fibrilla pairs (*cf.* LIMA-DE-FARIA, 1952). This fact allows a fairly accurate comparison between the standard fragment and its various derivatives.

In Fig. 21 such a comparison can be made between the standard fragment and the deleted fragment. The two chromosomes in question are the same as those in Figs. 2 and 15, but they have been straightened out and placed parallel to each other, allowing a direct comparison between the different regions. From this figure, as well as from the other chromosomes reproduced, it is quite clear that the main difference between the standard fragment and the deleted fragment is really represented by the terminal half of the long arm, which is absent in the deleted fragment.

In Fig. 21 differences between the short arms of the two chromosomes are also indicated but these are probably only apparent, the detailed appearance of the short arm being somewhat variable as may be seen from Figs. 1—20. Counts of the chromomeres were also undertaken, the following results being obtained (Table 2).

TABLE 2. *Number of chromomeres at pachytene in the deficiency fragment and the standard fragment.*

Deficiency fragment				Standard fragment				
Slide number	Chromosome figure No.	Number of chromomeres in short arm	long arm	Slide number	Chromosome figure No.	Number of chromomeres in short arm	long arm, region 1	long arm, region 2
1140	1	7	10	1112	15	9	13	17
»	2	6	11	»	16	10	14	19
»	3	7	10	»	17	8	11	15
1137	4	6	13	1033	18	5	11	18
»	5	7	10	»	—	8	14	18
»	6	7	11	1034	19	8	12	16
1131	7	6	11	1057	20	7	13	16
»	8	7	9	»	—	6	12	15
»	9	5	10					
888	10	7	11					
»	11	6	11					
889	12	5	10					
»	13	6	9					
»	14	6	10					
	Average:	6,3	10,4		Average:	7,6	12,5	16,8

In the short arm of the deleted fragment the number of chromomeres apparently ranged from 5 to 7 with an average number of 6,3. In the longer, deficient arm of the same chromosome variation was from 9 to 13 with an average of 10,4. In the centromere itself 1 or 2 chromomeres were seen in a few cases, but in the majority of the chromosomes studied no centromeric chromomeres could be distinguished.

In the standard fragments, studied for comparison, the number of chromomeres in the short arm ranged from 5 to 10 with an average of 7,6. In the long arm two regions may be distinguished, the first one representing the proximal part of the chromosome arm which is present also in the deleted fragment. This region had on an average 12,5 chromomeres. The second region, present in the standard fragment but absent in the deleted fragment contained on an average 16,8 chromosomes (Table 2).

In the deleted fragment both the short arm and the deficient arm apparently contained a slightly lower number of chromomeres than the corresponding regions in the standard fragment. With regard to the short arm the values were 6,3 and 7,6 respectively, the corresponding values of the other arm being 10,4 and 12,5. The two differences 7,6—6,3 and 12,5—10,4 were tested with an analysis of variance, both being found to be significant. In the first case v^2 will get the value 7,36 corresponding

to a P intermediate between 0,05 and 0,01. In the latter case v^2 was found to be 18,67 and P smaller than 0,001.

Most probably these differences do not represent structural differences between the chromosome regions in question but only signify that time and conditions of fixation or staining have been slightly different in the two groups of material which were fixed in different years. Most important is the fact that the fixations of the deficiency fragment represent a pachytene stage slightly later than those of the standard fragment. Therefore, owing to the chromosome contraction during prophase a certain number of chromomeres are more close to each other than the corresponding ones in the standard fragment. This explanation is in agreement with the fact that the ratio between the two chromosome regions is almost exactly the same in the deleted fragment and the standard fragment ($10{,}4 : 6{,}3 = 1{,}65$ in the former case, and $12{,}5 : 7{,}6 = 1{,}64$ in the latter case).

From the analysis undertaken here it is clear that the accessory chromosome, so far called a »deleted fragment», really does not represent a deletion but a terminal deficiency, deletions by definition representing intercalary losses of segments. Thus, it is necessary in further work to change the denomination »deleted fragment» to »deficient fragment» or »deficiency fragment».

Contrary to expectation the segment lacking in this chromosome is not the particular segment in the long arm close to the centromere, which was supposed to be primarily responsible for the non-disjunction-process shown by the standard fragment and the large iso-fragment. This segment must be present in the deficiency fragment as well, but in this chromosome it is not involved in any-disjunction. Thus, some other factor must be responsible for the occurrence of non-disjunction in some types of accessory chromosomes and its absence in other types.

Only two alternatives of explanation seem to be possible. The first one would be that the absolute size of the chromosomes in question determines their ability of undergoing non-disjunction, large iso-fragments and standard fragments being larger than the deficiency fragment and the small iso-fragment. This explanation, however, does not seem very plausible as the difference between ability and absence of non-disjunction is quite sharp. If absolute size were the decisive factor, a gradient in frequency of non-disjunction would be expected among the four types of B-chromosomes rather than two clear-cut categories.

The other alternative implies that the whole or part of the chromosome segment lacking in the deficiency fragment is responsible for

the occurrence of non-disjunction. This segment is also absent in the small iso-fragment but present in the standard fragment and the large iso-fragment. In such a case it must be assumed that this segment, though not itself mechanically involved in the non-disjunction process, is able to influence the behaviour of other parts of the same chromosome. As a working hypothesis it may be assumed that this is brought about by the passage of some substance along the chromosome, either from the »deficiency segment» to the »non-disjunction segment» or inversely. This suggestion is somewhat supported by the demonstration by RHOADES (unpublished; *cf.* RHOADES and KERR, 1949) that the formation of subsidiary spindle fibres from the terminal part of a chromosome arm in *Zea* is controlled by the localized centromere, situated far from this chromosome end.

With regard to our material it is tempting to assume that the conspicuous knob formation situated near the end of the long arm may be involved in the control of the non-disjunction process, this knob formation being present in the B-chromosomes showing non-disjunction and absent in the B-chromosome types lacking this ability. However, more information is evidently needed from other derivatives of the standard fragment before definite conclusions can be drawn. Some new derivatives of this kind have already been obtained.

It should be pointed out that the deficiency fragment represents a new case of »healing» of a broken chromosome end of the kind discovered by MCCLINTOCK (1941) in *Zea*. In the present case the new end of the deficient chromosome shows no indication of sister-reunion or other irregularities, and from the chromomere analysis it is quite clear that the structural change represents a true deficiency and not an intercalary deletion.

It should be remarked that the breakage took place in a region of the chromosome representing a conspicuous natural landmark used as the limit between Divisions 52 and 53 of the chromosome map of the standard fragment (LIMA-DE-FARIA, 1952). RANDOLPH (1941) pointed out that breaks in the B-chromosome derivatives in maize tend to occur more frequently at certain points than at others.

Finally it should be observed that the standard fragment and its derivatives are all subinert (MÜNTZING, 1943). Thus, the loss of large segments of the standard fragment does not involve a loss of vigour or fertility, plants with one or two deficiency fragments being equally, or more, vigorous and fertile than plants with one or two standard fragments.

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PREPARATORY STUDIES FOR BREEDING ICELANDIC POA IRRIGATA

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I. INTRODUCTION.

THE present writer has had some possibilities to make preparatory studies for future plant breeding work with forage and pasture crops in Iceland during the past five years. As the experimental facilities were minute and uncertain, all experiments made were of a rather preliminary character. Cytological studies on the material were, however, rather complete, and the studies on flower biology and the mechanism of seed formation of most of the species investigated gave results showing the most useful methods of breeding the different species.

One of the most valuable plants of the Icelandic cultivated grasslands is the species *Poa pratensis* L. in its widest sense. In northern Europe it has been found to be taxonomically classifiable in four subspecies, *viz.* ssp. *eu-pratensis* HIITT., ssp. *angustifolia* (L.) LINDB. FIL., ssp. *irrigata* (LINDM.) LINDB. FIL., and ssp. *alpigena* (FR.) HIITT. (*cf.* HIITONEN, 1933; HYLANDER, 1941, 1945), which according to other taxonomists (*cf.* HULTÉN, 1950; SELANDER, 1950) might more correctly be regarded as separate species. For the sake of simplicity the present writer wishes to use species names for these four types, although he does not defend any definite point of view in this taxonomical question at the time. The group belongs to the section *Stoloniferae* NANNF. of the genus, and besides *Poa irrigata* and *P. alpigena* it is represented in Iceland only by one other indigenous species, *i.e.* the arctic-montane species *P. arctica* R. BR. (*cf.* LÖVE, 1947; LÖVE and LÖVE, 1948; NYGREN, 1950).

Seeds available for agricultural needs of stoloniferous *Poae* seem to belong almost exclusively to the species *P. pratensis* in its strict sense, and most of the strains seem to originate from cultivations in North America, where the species has probably been introduced by man rather early. In Iceland different strains have been used during the last decades,

This paper was prepared at the University Institute of Applied Sciences, Reykjavík, Iceland.

but they have almost always been found to be inferior to the wild types of *P. irrigata* and *P. alpigena,* which in few years after the re-seeding of the grasslands are found re-established in the pastures. Strains of typical *P. pratensis* are, therefore, rather rare in the cultivated fields.

Due to the high competitive value of the Icelandic strains of *P. irrigata* and *P. alpigena* it was assumed to be of great value for Icelandic agriculture if at least the former and more common species could be bred and cultivated for seeds on such a large scale to make all importance of foreign strains of *P. pratensis* unnecessary. The present writer began in 1946 a rather extensive investigation, therefore, which will be described in more detail in the present paper as its results might be of some value for other breeders of the genus *Poa.* Though some of the results might seem to be rather fragmentary, they have to be published now as the experiments cannot be continued by the writer, who is moving from Iceland.

It is known from different species of the genus *Poa* that its basic number of chromosomes is $x = 7$ (*cf.* LÖVE and LÖVE, 1948; TISCHLER, 1950). Within the section *Stoloniferae*, however, a remarkable range of euploid as well as aneuploid chromosome numbers has been shown to occur in populations from different parts of its area of distribution. The lowest number yet known for the section seems to be $2n = 28$, but the highest number hitherto published was $2n =$ c. 124 (*cf.* NYGREN, 1950). Numbers between 50 and 100 are fairly common in *P. pratensis* s. lat. and represent an almost complete aneuploid series (*cf.* ÅKERBERG, 1942; ANDERSON, 1927; ARMSTRONG, 1937; BRITTINGHAM, 1941, 1943; BROWN, 1939, 1941; BÖCHER and LARSEN, 1950; FLOVIK, 1938, 1940; HARTUNG, 1946; KRAMER, 1947; LÖVE and LÖVE, 1942; MÜNTZING, 1933, 1937, 1938, 1940; NIELSEN, 1946; NISSEN, 1950; NYGREN, 1950; RANCKEN, 1934; TINNEY, 1940; *cf.* also TISCHLER, 1950, for further references).

As to the different species of the section, the widest range of chromosome numbers has been found within *P. pratensis* s. str., or the numbers $2n = 28$—124. This species has also been studied far more intensively than the others. Within *P. angustifolia* only the numbers $2n = 50$, 64, and 65 have been reported (ÅKERBERG, 1942), while *P. alpigena* is characterized by numbers between $2n = 35$ and 92 (ÅKERBERG, 1942; FLOVIK, 1938, 1940; HARTUNG, 1946; NYGREN, 1950; SÖRENSEN and WESTERGAARD in LÖVE and LÖVE, 1948, and in NYGREN, 1950). The only numbers hitherto published for *P. irrigata* seem to be $2n = 48$ (NYGREN in LÖVE and LÖVE, 1948) and $2n =$ c. 84—101 (ÅKERBERG, 1942). The different types of *P. arctica* are found to have numbers varying between

$2n = 39$ and c. 100 (ARMSTRONG, 1937; FLOVIK, 1938, 1940; NANNFELDT, 1940; NYGREN, 1950; SÖRENSEN and WESTERGAARD in LÖVE and LÖVE, 1948). Varying euploid as well as aneuploid numbers are also reported by HARTUNG (1946) for the American species *P. arachnifera* TOBR., *P. arida* VASEY, *P. glaucifolia* SCRIBN. et WILL., and *P. nervosa* (HOOK.) VASEY. The hexaploid number $2n = 42$ characterizes *P. confinis* VASEY (HARTUNG, 1946), while the tetraploid number $2n = 28$ is typical for *P. Douglasii* NEES, *P. macrantha* VASEY, and *P. rhizomata* HITCHC. (HARTUNG, 1946).

As the aneuploid numbers within this group were found to be fairly frequent and constant, MÜNTZING (1933) suggested that the reproductive basis for this phenomenon must be the occurrence of apomixis. Later on ÅKERBERG (1936, 1938, 1939, 1942, 1943), BRITTINGHAM (1941), ENGELBERT (1940, 1941), NILSSON (1937), NYGREN (1950), and TINNEY (1940) demonstrated that pollination is necessary for seed development of these types and that the seed formation itself is based upon apospory (*cf.* GUSTAFSSON, 1946, 1947 a, b).

Although it seems to be generally accepted that the seed formation at least of *P. pratensis* should be dependent on apospory, several workers have clearly demonstrated that it is not complete in all strains and that in some strains it might be combined with normal sexual seed formation in up to 50 per cent of the strains. By a simple progeny test it has been shown that although the predominating type of progeny is certainly identical with the mother plant and, thus, associated with apomictic seed formation, variant progeny is met with in some frequency. The deviating individuals are supposed to have been produced by some type of normal sexual reproduction, a supposition supported by cytological and embryological studies. MÜNTZING (1937, 1938) found that 9 per cent of twin plants of *P. pratensis* investigated by him showed deviations in the number of chromosomes, while ÅKERBERG (1939, 1942) found that approximately 7 per cent aberrants were met with in normal seedlings and about 13 per cent deviating individuals in material originating from twins. In 37 out of 44 families the progeny was completely uniform. NISSEN (1950) counted about 10 per cent aberrants from normal seedlings and approximately 30 per cent from twins in material from southwestern Norway.

Studies on morphologically aberrant progenies have been made most extensively by American workers. One of the most extensive investigations was performed by TINNEY and AAMODT (1940). They studied 102 progenies grown from seeds collected from single open-pollenated

panicles. In the total progenies, only 1,65 per cent of the plants were aberrants, but in 48 out of the 102 progenies not a single variant was observed. The highest frequency of variants was 21,9 per cent in one progeny. As cytological studies do not appear to have been undertaken on this material, no accounts as to the correlation between the degree of apomixis and the number of chromosomes are mentioned by TINNEY and AAMODT (*l. c.*), but in material studied by BROWN (1941) apomixis was found to be prevalent within the strains characterized by the highest number of chromosomes. In his material the frequency of aberrant types was found to range between 0,69 and 18,18 per cent.

The material investigated by TINNEY and AAMODT (1940) was studied in succeeding generations by SMITH and NIELSEN (1945). Their material consisted of 45 families which were studied for four generations. In all, about 11.400 individuals were observed. Although progenies from plants classified as aberrant were generally higher in proportions of deviating types than the normal sister plants, selection for normal and apomictic types did not appear to be highly effective as to the elimination of the tendency towards sexual reproduction. Even progenies of families classified as completely apomictic were found to show some low frequency of deviations in some later generations, and none of the families showed complete uniformity for two successive generations. The seven most constant families studied were found in the first, second, and third generations to show percentages of normal plants ranging between 59 and 100 per cent, the most constant type showing 98, 91, and 100 per cent of maternal individuals in three successive generations. SMITH, NIELSEN, and AHLGREN (1946) reported that as high a frequency as 16,5 per cent of aberrant plants was met with in their material.

Studies on the variation in the progeny of *P. pratensis* have also been performed by BRITTINGHAM (1943), who studied the progeny of 115 plants and found 14,6 per cent aberrants in all the material, while the values for different families ranged from zero to 65,5 per cent. This high per cent of variability might be caused by the method used in the investigation, as all the seeds were germinated in Petri dishes permitting equal chances for survival. This suggestion is supported by the observation that a highly significant negative correlation was found between survival and variability. MYERS (1943) made further tests on three of these progenies in a second generation, and his results agreed in general with the results obtained by BRITTINGHAM (*l. c.*), although some minor errors in classification were detected. Variant-type individuals were found to produce a higher proportion of sexual seeds than did

their parents or parental-type families. From this it was concluded that »the first-generation test provided a satisfactory preliminary evaluation of method of reproduction» (*cf.* ATWOOD, 1947) for plant-breeding works with *P. pratensis*.

Although morphological variability might be rather distinct in material from the same locality, due to the variation in chromosome number and degree of apomixis, not all authors agree whether differences in yield of the different progenies are really met with. AHLGREN, SMITH, and NIELSEN (1945) found little or no correlation between yields of spaced plants and their clonal progeny or between yielding ability and disease reaction, while BRITTINGHAM (1943), MYERS and GARBER (1942), MYERS and SPRAGUE (1944), and SPRAGUE and MYERS (1945) reported differences in yields of clones under clipping at least when grown in association with white clover. HAYES and THOMAS (1944) and THOMAS and HAYES (1947) detected little or no relationship between second year's average yields and the first year yields. MÜNTZING (1940) found some little difference in yield between »diploid» and »triploid» twins in the third year, while NISSEN (1950) found no significant correlation between chromosome number and the evaluation of the individuals as pasture plants. BROWN (1939) found that wide panicles, large spikelets, increased number of florets per spikelet, as well as long and numerous rhizomes tend to be associated with wide leaves, and that wide-leaved individuals are generally low polyploid. He also observed a decline in vigour when the chromosome number exceeded the hexaploid condition. NISSEN (1950), however, did not obtain any significant correlation between chromosome number and winter killing, number of culms, culm thickness, seed set, pollen quality, seed germination, and number of nodes in the panicle, while positive correlation was observed between chromosome number and culm height as well as number of spikelets. MÜNTZING (1940) and ÄKERBERG (1942) found the plants with the lower numbers of chromosomes to be taller than those with the higher numbers, while KRAMER (1947) found a low, though non-significant, positive correlation between height and chromosome number in his material of clones with chromosome numbers varying between $2n = 50$ and 88. KRAMER (*l. c.*) concluded from his investigations that although chromosome numbers were positively associated with spreading rate and with mildew susceptibility, »chromosome numbers in Kentucky bluegrass may vary over a considerable range without having an appreciable affect on morphologic characters or agronomic behavior». As most of the investigators referred to above made no distinctions be-

tween the euploid and aneuploid series in their material when checking correlations between chromosome numbers and other characters, this conclusion is perhaps somewhat superficial, especially as it is well-known from other plant genera that aneuploidy might affect the vigour and viability of the individual itself.

Based on his knowledge of *P. pratensis* s. lat. as referred to above the present writer planned his studies on the Icelandic *P. irrigata*. As only a few counts of its chromosome numbers had been made previously, a huge material was studied in order to check if its variations were the same as in *P. pratensis* s. str., and in connection to these studies investigations were performed on the possible correlation of different morphological characters with the number of chromosomes as well as on the occurrence of aberrations from the apomictic or apogamous seed formation which was already known to occur within this species (ÅKERBERG, 1942).

II. MATERIAL AND METHODS.

The material used in this investigation was collected in a rather large number of localities in different parts of Iceland during the summers of 1946 to 1950. In most of the localities some few plants were collected, and the sod pieces were broken down completely by aid of water in order to ascertain that only one single individual was included. In some of the cases fixations were made immediately, while other individuals were cultivated in pots at the farm Ulfarsá near Reykjavík and fixed there. The morphological studies were made only on the potted material, but due to lack of facilities it was impossible to cultivate the plants in the field without the possibility of including contaminating weeds of the same species as it is found to be very common in S. W.-Iceland. Therefore, no progeny tests could be made outside the laboratory. Owing to lack of greenhouses the potted plants had to survive the winters in the pots, but due to circumstances not under the control of the writer the winter survival of the different strains could not be calculated without great uncertainty.

For chromosome counts the root-tips were fixed in the Svalöf modification of Navashin's fixation (*cf.* MÜNTZING, 1933), and the 13 microns thick sections were stained according to the crystal violet method (*cf.* DARLINGTON and LA COUR, 1942), and mounted in Canada-balsam. The morphological fertility of the pollen grains was studied in acetocarmine-glycerine.

III. CHROMOSOME NUMBERS.

As mentioned above, the chromosome numbers known within the species *P. pratensis* s. str. are found to vary betweeen $2n = 28$ and c. 124, values below 49 and above 91 being exceptional. All values between 49 and 91 seem to be met with, but according to BROWN (1939)

TABLE 1. *Chromosome numbers of Icelandic Poa irrigata.*

Chromosome number, 2 *n*	S.-Iceland	S. W.-Iceland	W.-Iceland	N.-Iceland	Total	Chromosome number, 2 *n*	S.-Iceland	S. W.-Iceland	W.-Iceland	N.-Iceland	Total
82	1	1	1	—	3	*116*	—	5	—	—	5
83	—	—	1	—	1	*117*	—	2	—	1	3
84	13	21	16	—	50	*118*	2	10	—	—	12
85	1	—	—	—	1	*119*	3	83	13	6	105
86	—	—	1	—	1	*120*	1	2	—	—	3
87	1	1	—	—	2	*121*	—	3	—	1	4
88	—	1	—	—	1	*122*	—	2	—	1	3
89	1	—	1	—	2	*123*	—	—	1	—	1
90	1	—	—	—	1	*124*	—	1	—	1	2
91	5	14	5	—	24	*125*	—	—	—	1	1
92	1	—	—	—	1	*126*	1	6	11	17	35
93	—	1	1	—	2	*127*	—	—	1	1	2
94	1	—	1	—	2	*128*	—	1	—	1	2
95	—	2	—	1	3	*129*	—	—	—	1	1
96	—	—	2	—	2	*130*	—	2	—	—	2
97	1	1	—	—	2	*131*	—	—	1	—	1
98	9	39	8	4	60	*132*	1	—	—	—	1
99	1	—	1	—	2	*133*	2	11	4	3	20
100	—	2	—	1	3	*134*	—	—	1	—	1
101	1	6	—	1	8	*135*	—	1	—	—	1
102	—	1	1	—	2	*136*	—	—	1	1	2
103	—	1	—	—	1	*137*	—	4	—	—	4
104	—	2	—	—	2	*138*	—	—	2	—	2
105	4	72	6	2	84	*139*	—	1	—	1	2
106	1	3	—	—	4	*140*	—	16	19	17	52
107	—	1	1	1	3	*141*	—	—	1	1	2
108	—	3	1	—	4	*142*	—	1	—	1	2
109	—	4	—	—	4	*143*	—	—	1	—	1
110	—	2	—	1	3	*144*	—	—	2	1	3
111	1	1	1	—	3	*145*	—	—	1	—	1
112	7	102	3	15	127	*146*	—	1	—	1	2
113	—	2	—	1	3	*147*	—	—	—	2	2
114	—	3	1	1	5	*Total*:	60	440	112	88	700
115	—	2	1	1	4						

and VINALL and HEIN (1937) there is a strong mode at the octaploid number $2n = 56$. The variations known from other apomictic species of *Poa* seem also to lie within these limits, although much fewer counts have been made in other species.

TABLE 2. *Chromosome numbers of Poa irrigata at different altitudes.*

Chromosome number, 2 *n*	S.W.-Iceland		W.-Iceland		N.-Iceland		Chromosome number, 2 *n*	S.W.-Iceland		W.-Iceland		N.-Iceland	
	Ulfarsá, ca. 50 m. s. m.	Mosfellsheiði, ca. 360 m. s. m.	Andakíll, ca. 30 m. s. m.	Holtavörðuheiði, ca. 300 m. s. m.	Eyjafjörður, ca. 20 m. s. m.	Vaðlaheiði, ca. 400 m. s. m.		Ulfarsá, ca. 50 m. s. m.	Mosfellsheiði, ca. 300 m. s. m.	Andakíll, ca. 30 m. s. m.	Holtavörðuheiði, ca. 300 m. s. m.	Eyjafjörður, ca. 20 m. s. m.	Vaðlaheiði, ca. 400 m. s. m.
82	—	—	1	—	—	—	*116*	4	—	—	—	—	—
83	—	—	1	—	—	—	*117*	2	—	—	—	1	—
84	5	—	16	—	—	—	*118*	9	1	—	—	—	—
85	—	—	—	—	—	—	*119*	61	7	—	13	4	2
86	—	—	1	—	—	—	*120*	2	—	—	—	—	—
87	—	—	—	—	—	—	*121*	3	—	—	—	1	—
88	—	—	—	—	—	—	*122*	1	1	—	—	—	1
89	—	—	1	—	—	—	*123*	—	—	—	1	—	—
90	—	—	—	—	—	—	*124*	1	—	—	—	1	—
91	3	—	5	—	—	—	*125*	—	—	—	—	—	1
92	—	—	—	—	—	—	*126*	5	1	—	11	10	7
93	1	—	1	—	—	—	*127*	—	—	—	1	—	1
94	—	—	1	—	—	—	*128*	—	1	—	—	1	—
95	1	—	—	—	1	—	*129*	—	—	—	—	—	1
96	—	—	2	—	—	—	*130*	—	2	—	—	—	—
97	1	—	—	—	—	—	*131*	—	—	—	1	—	—
98	33	1	8	—	4	—	*132*	—	—	—	—	—	—
99	—	—	1	—	—	—	*133*	—	11	—	4	—	3
100	2	—	—	—	1	—	*134*	—	—	—	1	—	—
101	4	1	—	—	1	—	*135*	—	1	—	—	—	—
102	1	—	1	—	—	—	*136*	—	—	—	1	—	1
103	1	—	—	—	—	—	*137*	—	4	—	—	—	—
104	2	—	—	—	—	—	*138*	—	—	—	2	—	—
105	46	6	4	2	2	—	*139*	—	1	—	—	—	1
106	3	—	—	—	—	—	*140*	—	16	—	19	—	17
107	1	—	—	1	1	—	*141*	—	—	—	1	—	1
108	1	—	1	—	—	—	*142*	—	1	—	—	—	1
109	3	1	—	—	—	—	*143*	—	—	—	1	—	—
110	1	—	—	—	1	—	*144*	—	—	—	2	—	1
111	—	—	—	1	—	—	*145*	—	—	—	1	—	—
112	89	2	—	3	14	1	*146*	—	1	—	—	—	1
113	1	—	—	—	1	—	*147*	—	—	—	—	—	2
114	3	—	—	1	1	—							
115	1	1	—	1	—	1	*Total*:	291	60	44	68	45	43

In order to get reliable informations as to the chromosomal variation within the Icelandic types of *P. irrigata*, material was collected in different parts of the country. As in *P. pratensis* the chromosome numbers were found to vary considerably, but the numbers were higher, or only within the range between $2n = 82$ and 147, as shown in Table 1. The numbers are found to be scattered between these two limits, but although all numbers within this range are represented, distinct peaks seem to be met with at the euploid numbers $2n = 84$ (12×7), 91 (13×7), 98 (14×7), 105 (15×7), 112 (16×7), 119 (17×7), 126 (18×7), 133 (19×7), and 140 (20×7). The most frequent euploid numbers are $2n = 112$ and $2n = 119$ with 127 and 105 individuals, respectively.

Although the material is perhaps not representative for all the variations in chromosome number within *P. irrigata* in different regions, as it was not collected in as large number of localities in all the parts of the country given in Table 1, it might be of some interest to point out that the lowest numbers, or $2n = 82$—94, are not found in individuals from the northern parts of the country, while numbers higher than $2n = 133$ have not yet been detected in material from southern Iceland. This might, of course, be due to some coincidence, as the material from northern Iceland is rather scanty. The present writer is, however, inclined not to regard it as a mere chance that in southwestern, western, as well as in northern Iceland higher chromosome numbers are met with at higher altitudes, as shown in Table 2. Although not being fit for closer statistical analysis the tendency shown in all this material clearly indicates that the types with the higher numbers of chromosomes should be better adapted for the conditions in the mountains than those with lower numbers. This is also in concordance with the hypothesis of the positive correlation between chromosome number and ability of growing at high latitudes and altitudes (*cf.* MÜNTZING, 1936; LÖVE and LÖVE, 1949).

As rather wide variations were observed in the material collected in the locality Kópavogur during the first summer of observations, closer studies were made on the chromosome number of individuals collected within a small and very restricted area. At the same time as such studies might give informations about the chromosomal variation between plants growing side by side, they will also give some measurement as to the number of strains growing in the same field and the competitive power of some of the strains. In Kópavogur all individuals of *P. irrigata* within each of three rings with 10 metres diameter were studied as to their chromosome number, but the number of rings with 10 metres

TABLE 3. *Chromosome numbers of Poa irrigata within rings of 10 m diameter.*

Chromosome number, 2 *n*	Kópavogur			Ulfarsá					
	First ring	Second ring	Third ring	First ring	Second ring	Third ring	Fourth ring	Fifth ring	Sixth ring
82	—	1	—	—	—	—	—	—	—
83	—	—	—	—	—	—	—	—	—
84	12	4	—	2	—	—	—	3	—
85	—	—	—	—	—	—	—	—	—
86	—	—	—	—	—	—	—	—	—
87	—	1	—	—	—	—	—	—	—
88	1	—	—	—	—	—	—	—	—
89	—	—	—	—	—	—	—	—	—
90	—	—	—	—	—	—	—	—	—
91	6	2	3	1	2	—	—	—	—
92	—	—	—	—	—	—	—	—	—
93	—	—	—	—	—	—	—	1	—
94	—	—	—	—	—	—	—	—	—
95	—	1	—	—	1	—	—	—	—
96	—	—	—	—	—	—	—	—	—
97	—	—	—	—	—	1	—	—	—
98	3	1	1	7	11	4	5	1	—
99	—	—	—	—	—	—	—	—	—
100	—	—	—	—	—	—	2	—	—
101	1	—	—	—	—	1	—	—	—
102	—	—	—	—	1	—	—	—	—
103	—	—	—	1	—	—	—	—	—
104	—	—	—	—	—	1	1	—	—
105	7	11	2	—	3	16	3	8	1

Chromosome number, 2 *n*	Kópavogur			Ulfarsá					
	First ring	Second ring	Third ring	First ring	Second ring	Third ring	Fourth ring	Fifth ring	Sixth ring
106	—	—	—	—	1	—	2	—	—
107	—	—	—	1	—	—	—	—	—
108	—	1	1	—	—	1	—	—	—
109	—	—	—	—	1	—	—	2	—
110	1	—	—	—	—	—	1	—	—
111	—	1	—	—	—	—	—	—	—
112	3	4	4	14	6	7	16	2	5
113	—	1	—	—	—	—	—	1	—
114	—	—	—	—	—	2	—	1	—
115	—	—	—	—	1	—	—	—	—
116	—	—	1	2	—	—	—	—	1
117	—	—	—	—	—	1	1	—	—
118	—	—	—	—	—	—	1	1	—
119	—	1	14	5	3	11	10	2	14
120	—	—	—	—	—	—	2	—	—
121	—	—	—	—	—	1	1	—	—
122	—	—	—	—	—	—	—	—	1
123	—	—	—	—	—	—	—	—	—
124	—	—	—	—	—	—	—	—	1
125	—	—	—	—	—	—	—	—	—
126	—	—	—	—	—	—	1	1	3
Total:	34	29	26	33	30	46	46	23	26

diameter was six at Ulfarsá. The results obtained are shown in Table 3. It can be seen there that in every ring some different chromosome numbers are represented. In two of the rings the number of strains differing in chromosome number is seven, but in the other rings a still higher number of strains is met with.

In order to get informations about the number of strains in a still more restricted area, all individuals within each of five rings with 2 metres diameter were studied as to their chromosome number. The species *P. irrigata* is able to spread vegetatively considerably by aid of stolons, but in normal grasslands where the competition between individuals of the same species is fairly great, this ability seems to be

TABLE 4. *Chromosome numbers of Poa irrigata within rings of 2 m diameter.*

Chromosome number, 2 *n*	First ring	Second ring	Third ring	Fourth ring	Fifth ring	Chromosome number, 2 *n*	First ring	Second ring	Third ring	Fourth ring	Fifth ring
98	—	—	—	—	5	*111*	—	—	—	—	—
99	—	—	—	—	—	*112*	6	9	7	15	9
100	—	—	—	—	—	*113*	—	—	—	—	—
101	—	1	—	—	2	*114*	—	—	—	—	—
102	—	—	—	—	—	*115*	—	—	—	—	—
103	—	—	—	—	—	*116*	—	1	—	—	—
104	—	—	—	—	—	*117*	—	—	—	—	—
105	—	2	1	—	12	*118*	3	1	2	—	—
106	—	—	—	—	—	*119*	1	6	4	9	1
107	—	—	—	—	—	*120*	—	—	—	—	—
108	—	—	—	—	—	*121*	—	—	1	—	—
109	—	—	—	—	—	*Total:*	10	20	15	24	29
110	—	—	—	—	—						

somewhat suppressed. The results of the studies on the material from the smaller rings are given in Table 4. Although not a single ring was found to be inhabited only by one strain with the same chromosome number, all the rings include only 2—4 different numbers. It might be concluded, therefore, that it should be necessary to collect individuals somewhat far apart if samples of different chromosome numbers of *P. irrigata* are to be collected within a restricted area. In all common Icelandic home fields of the normal type great variations in chromosome number of *P. irrigata* might be expected to occur.

IV. SEED FORMATION.

As pointed out in the introductory chapter of this paper, several authors have shown that studies on the seed formation of the stoloniferous *Poae* might be made indirectly through studies on the morphological and cytological variability of the progeny of the different plants. As some of the aberrant individuals might be less compatible in nature, the only completely reliable method for detecting all the variability in chromosome number should be that based on cytological studies on all the germinating seeds. In that case only the already dead seeds are not checkable.

TABLE 5. *Chromosome numbers of offspring of Poa irrigata.*

Chromosome numbers of offspring	Chromosome numbers of mother plants																		
	2 *n* = 84	2 *n* = 84	2 *n* = 84	2 *n* = 87	2 *n* = 91	2 *n* = 98	2 *n* = 101	2 *n* = 107	2 *n* = 119	2 *n* = 126	2 *n* = 129	2 *n* = 131	2 *n* = 133	2 *n* = 136	2 *n* = 139	2 *n* = 140	2 *n* = 140	2 *n* = 143	2 *n* = 147
82	2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
83	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
84	32	41	45	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
85	1	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
86	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
87	—	—	—	29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
88	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
89	—	—	—	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
90	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
91	—	—	—	1	37	—	—	—	—	—	—	—	—	—	—	—	—	—	—
92	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
93	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
94	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
95	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
96	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
97	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
98	—	—	—	—	—	42	1	—	—	—	—	—	—	—	—	—	—	—	—
99	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
100	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
101	—	—	—	—	—	1	37	—	—	—	—	—	—	—	—	—	—	—	—
102	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
103	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
104	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
105	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
106	—	—	—	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
107	—	—	—	—	—	—	—	29	—	—	—	—	—	—	—	—	—	—	—
108	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
109	—	—	—	—	—	1	—	2	—	—	—	—	—	—	—	—	—	—	—
110	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
111	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
112	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
113	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
114	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
115	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
116	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
117	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
118	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
119	—	—	—	—	—	—	—	—	33	—	—	—	—	—	—	—	—	—	—
120	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
121	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
122	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—

Chromosome numbers of offspring	Chromosome numbers of mother plants																			
	2 n = 84	2 n = 84	2 n = 84	2 n = 87	2 n = 91	2 n = 98	2 n = 101	2 n = 107	2 n = 119	2 n = 126	2 n = 129	2 n = 131	2 n = 133	2 n = 136	2 n = 139	2 n = 140	2 n = 140	2 n = 143	2 n = 147
123	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
124	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
125	—	3	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
126	—	9	—	1	—	—	—	—	—	42	1	—	—	—	—	—	—	—	—
127	—	1	—	3	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—
128	—	—	—	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
129	—	—	—	1	—	—	—	—	—	—	29	1	—	—	—	—	—	—	—
130	—	—	—	5	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—
131	—	—	—	1	1	—	—	—	—	—	1	32	—	—	—	—	—	—	—
132	—	—	—	—	—	—	—	—	—	—	1	2	1	2	—	—	—	—	—
133	—	—	—	—	—	—	—	—	—	—	2	1	36	1	—	—	—	—	—
134	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
135	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—
136	—	—	—	—	—	—	—	—	—	—	—	—	—	28	—	—	—	—	—
137	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
138	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
139	—	—	—	—	—	—	—	—	—	—	—	—	—	—	29	—	—	—	—
140	—	—	—	—	—	1	—	—	—	—	—	—	—	—	2	32	41	—	—
141	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—
142	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
143	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1	—	—	27	—
144	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
145	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
146	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—
147	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	39

The author has made chromosome counts on the seed progeny of 19 freely flowering individuals with different chromosome numbers. The mother plants were cultivated in pots at Ulfarsá, but the seeds were raised in the laboratory in Reykjavík in Petri dishes. The results obtained are given in Table 5.

Only four out of the 19 progenies studied were completely uniform as to their chromosome number. Most of the other progenies were found to show some small variations, which might be the results of uneven somatic divisions of the extra chromosomes. Such variations may or may not be morphologically distinguishable. In four progenies, however, some individuals were found to be characterized by such a wide variation in the number of chromosomes that it must be due to some irregularities in the apomictic behaviour of the mother plants making normal sexual seed formation possible. This was not observed

in progenies of plants with a number higher than $2n = 98$. As all individuals with $2n = 101$ or more chromosomes were found to leave progenies completely uniform or with only slight variations in chromosome number, the writer is inclined to suggest that the types with higher chromosome numbers should be characterized by more complete apomixis and parthenogenesis than types with lower numbers, as pointed out for *P. pratensis* by BROWN (1941).

It might be of some interest also to point out that although the present material is found to indicate the occurrence of semi-apomixis in some of the strains, as previously known from *P. pratensis* s. str. and *P. alpina* (*cf.* ÅKERBERG, 1939, 1942; HÅKANSSON, 1943, 1944; MÜNTZING, 1940), not a single really deviating number lower than that of the mother plant has hitherto been detected in *P. irrigata*. According to ÅKERBERG (*l. c.*), deviations are met with in his material of *P. pratensis* indicating a normal reduction division in the mother plant, then followed by normal fertilization or parthenogenetic seed formation resulting in individuals with distinctly lower chromosome numbers than typical for the mother plant itself. The chromosome numbers given in Table 5 of the present paper do not indicate that such a reduction takes place in *P. irrigata* in Iceland. On the other hand, the variations met with in the present material clearly indicate that although parthenogenesis is the dominant mode of reproduction in this species, it is not complete in all strains. But although fertilization sometimes takes place, all the present material supports the view that all the embryo-sacs of *P. irrigata* are formed exclusively by apospory, as the deviating numbers are found to be considerably higher than those of the mother plants.

V. MORPHOLOGICAL CHARACTERS.

As the material of *P. irrigata* hitherto studied was not grown in the field, observations on characters of the most agronomical value could not be made. Some of the characters studied on the potted individuals give, however, indications as to the probable agronomical value of the plants, although they were made mainly in order to get informations on the effect of the differences in chromosome number on these characters. All the results are summarized in Table 6, which gives only the mean values for each character and each strain as determined at Ulfarsá in 1948.

Pollen diameter. — The pollen diameter was measured on 200 grains from each plant, in acetocarmine-glycerine. It is found to in-

TABLE 6. *Morphological characters of strains with different chromosome numbers.*

Chromosome number	Pollen diameter	Stomata length	Pollen fertility	Seed setting	1000 seed weight	Germination of seeds	Green leaf mass	Leaf length	Culm height
$2n = 84$ (12x)	28,3 μ	51,2 μ	77 %	93 %	430 mg	82 %	265 g	15,3 cm	49,1 cm
$2n = 91$ (13x)	29,7 »	51,8 »	91 »	81 »	490 »	79 »	291 »	15,8 »	54,3 »
$2n = 98$ (14x)	30,2 »	52,3 »	98 »	96 »	515 »	88 »	341 »	16,5 »	58,9 »
$2n = 101$	31,1 »	51,8 »	73 »	80 »	519 »	73 »	311 »	15,3 »	52,6 »
$2n = 107$	32,5 »	52,4 »	81 »	84 »	529 »	78 »	302 »	15,7 »	54,1 »
$2n = 119$ (17x)	33,9 »	54,1 »	87 »	89 »	541 »	81 »	367 »	17,3 »	61,3 »
$2n = 126$ (18x)	35,1 »	56,3 »	97 »	92 »	587 »	91 »	399 »	17,6 »	68,8 »
$2n = 133$ (19x)	35,9 »	54,7 »	89 »	92 »	617 »	82 »	429 »	18,2 »	52,4 »
$2n = 139$	37,3 »	54,9 »	65 »	87 »	623 »	74 »	401 »	18,3 »	48,7 »
$2n = 140$ (20x)	37,4 »	56,4 »	96 »	98 »	652 »	93 »	485 »	18,8 »	42,5 »
$2n = 147$ (21x)	38,2 »	58,0 »	92 »	98 »	674 »	87 »	497 »	18,2 »	45,6 »

crease significantly with an increase in the number of chromosomes, as has previously been reported for *P. pratensis* by ÅKERBERG (1942), MÜNTZING (1940), and NISSEN (1950).

Stomata length. — The stomata length was measured on 200 stomata from 10 leaves of each plant, always from the under side of the leaves near the tip. As shown in Table 6, they are found to increase with an increase in chromosome number. ÅKERBERG (1942), MÜNTZING (1940), and NISSEN (1950) also made observations similar to this one for *P. pratensis*.

Pollen fertility. — The fertility of the pollen grains was studied only morphologically in acetocarmine-glycerine, where the filled grains were counted as normal. The fertility seems to be fairly good in all the samples, irrespective of the aneuploid and euploid variations in chromosome number. It cannot be excluded, however, that germination tests of the pollen grains would show considerable differences from the morphological test in *P. irrigata*, as previously shown, *e. g.*, for the four species of *Rumex* subg. *Acetosella* studied by LÖVE (1943).

Seed setting. — The seed setting was calculated as the number of seeds in percentage of number of flowers on the same culm. It was found to be fairly good on all the plants studied, although it shows some variations.

Seed weight. — The seed weight was calculated for 1000 seeds from five weighings of 250 seeds each from each plant. The increase in seed

weight is found to be clearly correlated to the increase in chromosome number, as previously found for *P. pratensis* by ÅKERBERG (1942), MÜNTZING (1940), and NISSEN (1950).

Seed germination. — In connection with the studies on the chromosome number of the seedlings, the germination percentage of the different strains was calculated. As shown in Table 6, it is found to vary rather widely, although no correlation between chromosome number and germination of the seeds is met with. The same has been reported for *P. pratensis* by NISSEN (1950).

Green leaf mass. — The green leaf mass of the potted plants was weighed twice during the summer. Although the weight might be rather uncertain in some cases and affected in different ways by the cultivation in pots, the tendency observable in Table 6 seems to support the view that the green weight of the plants is correlated with the increase in chromosome number.

Leaf length. — Thirty basal leaves of each individual were measured and their mean length calculated. The mean numbers are shown in Table 6. The leaf length of *P. irrigata* appears to be positively correlated with chromosome number.

Culm height. — As the measurements on the culm height were made on potted material only, rather great variations are met with in the same plant. Although the numbers given in Table 6 might indicate that the tallest plants should be the 18-ploid ones with about 126 chromosomes, the number for this strain is not necessarily significantly different from the lower means. The general tendency seems to be, however, towards a peak at the 18-ploid number with a decreasing curve towards the lower as well as the higher numbers of chromosomes. According to KRAMER (1947), a low positive correlation was found between height and chromosome number in his material of *P. pratensis* with $2n = 50$—88 chromosomes, while MÜNTZING (1940), ÅKERBERG (1942), and NISSEN (1950) found a negative correlation only between chromosome number and height of culms in their material of the same species.

VI. COMPREHENSIVE DISCUSSION.

Although the present investigation was planned mainly for practical purposes, its results are of theoretical interest as well. From the practical point of view it might give some indications as to the most reliable methods for plant breeding work with this species which has previously

not been bred for agricultural use. From the theoretical point of view, however, the results of the present studies show some interesting deviations from results previously obtained from related species although most of the major results coincide in full with those accumulated from these species.

As pointed out above, the seed formation of *P. pratensis* seems to be based mainly on apospory followed by pseudogamy, but sometimes normal sexual seed formation takes place, based on fertilization of aposporous eggs as well as of eggs with a reduced number of chromosomes. In the present rather extensive material of Icelandic *P. irrigata*, however, not a single case is met with indicating the occurrence of a reduction division at the forming of the egg cell. Fertilization of aposporous egg cells is, however, sometimes met with, as shown by the occurrence of deviating types with chromosome numbers distinctly higher than that of the mother plant. Such a fertilization seems to be met with in the individuals with the lower chromosome numbers only, as no indications of its occurrence in the individuals with the higher chromosome numbers have been as yet observed. From practical point of view this might be used in the breeding work, as it makes some controlled breeding by combination possible, although the degree of such fertilization is low. The minor variations in chromosome number in some of the offspring might be due to some irregularities at the somatic division of the extra chromosomes. Although perhaps morphologically recognizable, these latter types are of less value for the plant breeding work.

It has been stressed several times in the present paper that due to lack of facilities it was impossible to make all the experiments as extensive as should have been necessary if all the observations made had to become statistically significant. Nevertheless, these preliminary observations seem to indicate very clearly that rather simple breeding methods might give good results in the Icelandic material of *P. irrigata*. As the morphological characters of agronomical value already studied might seem to be somewhat positively correlated with rather high chromosome numbers, strains with high chromosome numbers should be collected and compared in the experimental field. The present observations also indicate that the types with the highest chromosome numbers should be expected to be more frequent in higher than in lower altitudes, especially in the northern parts of the country, so that collections from high altitudes might give better results than if the strains are taken only from the lowland. The variation in chromosome

number within restricted areas also indicates that a rather high frequency of different strains might be collected even at the same locality. When the agronomically best strains have been selected within this material with high chromosome numbers, it might also be expected that at least the great majority of the strains should be completely stabile, as the apomixis seems to be obligate in types with the higher numbers of chromosomes. Therefore, the plant breeding work with each strain with high number of chromosomes could be ended immediately after the completion of the selecting work, provided of course that the observations already made should be found to be general. As the Icelandic needs of seeds of *P. irrigata* seem to be only a few tons per year, it might take less than ten years to select strains and multiply them to fill the needs, especially if modern facilities for the selection and seed cultivation will be made available in the country.

From the theoretical point of view some of the observations made show the same features as previously known from species closely related to *P. irrigata*, while other indicate some dissimilarities. The chromosome number is found to vary considerably within this species as well as within some other species of *Poa*, but the numbers are as a whole higher than counted previously. They are found to cover the whole series from $2n = 82$ to 147, but only about 20 per cent of the 700 plants studied are characterized by aneuploid numbers. Distinct peaks are observed at all the euploid multiples of the basic number of *P. irrigata*, while no such peaks seem to be met with in the closely related *P. pratensis*, although a complete series of euploid and aneuploid numbers has been studied within that species also. This latter case could, however, be a coincidence only.

As the material used in the present investigation was studied first and foremost from practical aspects, closer observations on its characters of taxonomical value were not made. The characters used for separation of this unit from other apomictic groups of the genus *Poa* were, however, found to be stable, but some minor differences certainly appropriate for separating at least the euploid types were observed but not worked out more closely. These observations indicate, however, that combined cytological and taxonomical investigations on extensive material of the apomictic species of *Poa* might enable us to detect characters making it possible to classify the strains with the higher numbers of chromosomes already in their natural habitats. Such studies would be not only of theoretical interest, therefore, but of a considerable value for plant breeders working with the genus.

SUMMARY.

(1) The chromosome number was determined for 700 strains of *Poa irrigata* from different parts of Iceland. All numbers between $2n = 82$ and 147 were observed. Only 20 per cent of the plants were aneuploid, and distinct peaks were observed at all the euploid multiples. The numbers $2n = 112$ and 119 were found to be most frequent, represented by 127 and 105 individuals, respectively.

(2) In the northern parts of the country higher chromosome numbers are found than in the southern parts, and the same holds true for higher altitudes as compared with the lowlands in all parts studied.

(3) The variation in chromosome number is found to be considerably even in restricted areas, as shown by observations on all individuals within rings with 10 and 2 metres diameter, respectively.

(4) Studies on all the seeds from individuals with different chromosome numbers indicate that although the normal seed formation in *P. irrigata* is apospory followed by pseudogamy, deviations are met with showing the occurrence of fertilization with some low frequency. All the embryo-sacs seem, however, to be formed by apospory without reduction division. In strains with a high number of chromosomes parthenogenesis of the aposporous embryo-sacs seems to be obligate.

(5) A positive correlation between chromosome number and pollen diameter, stomata length, seed weight, green weight, and perhaps even culm height, seems to be met with, while no correlation is found between chromosome number and morphological pollen fertility, seed setting, and seed germination.

(6) Methods for plant breeding of *P. irrigata* are outlined. Selection of types with high chromosome numbers is supposed to be satisfactory for getting stable strains giving good yields of hay.

(7) Some observations made indicate that it might be possible to detect morphological differences between the euploid types of *P. irrigata*. If correct, this might be of a considerable theoretical as well as practical interest.

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HETEROSIS IN F_1 COMBINATIONS IN GALEOPSIS. I

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I. INTRODUCTION.

THIS paper presents the results of studies of the F_1 generation of different combinations between pure lines of the species *Galeopsis speciosa*, *pubescens*, *bifida*, and *Tetrahit*.

In a series of papers MÜNTZING (1927—1938) has described these lines and given an analysis of their genetical constitution; among other things he has demonstrated the occurrence of partial sterility in F_1 of certain combinations between pure lines within the species *Tetrahit*. He has also found several cases of evident »hybrid vigor» (1930 a) and in one paper (1945) has published data which indicate a relationship between the degree of pollen fertility and that of »hybrid vigor» in different F_1 combinations.

This encouraged further investigation, and in 1945 a series of studies was started with the intention of comparing the F_1 generation with the different parental pure lines of the *Galeopsis* species mentioned. These studies were part of a more general investigation, including also some other genera. The results obtained with the latter will be published separately.

At the start of the investigation the intention of the author was to try to find an answer to the following questions: (1) Where is hybrid vigor to be found? In which of the species and genera studied, and with special regard to *Galeopsis*, in which species and in which combinations within or between the species? (2) Is the hybrid vigor equally marked in diploid and in tetraploid species? (3) Is there any relation between the degrees of geographical and ecological differentiation between the parent lines and the degree of hybrid vigor in F_1? (4) Is there any relation between the degree of the reduction of fertility and that of hybrid vigor in F_1? (5) At which stages of development and in which characters does hybrid vigor become evident? (6) Is F_1 more variable than the pure lines? A seventh problem was also included in the programme, *viz.*, the occurrence of monofactorial hybrid vigor, but this

has been dealt with in other genera, the results to be published and discussed elsewhere.

It was desirable to study the largest possible number of combinations and the work had therefore to be confined to a study of the F_1 generation only, the working facilities not permitting a further study of F_2, F_3 or back-crosses. It has therefore been impossible to relate the material to the works of POWERS (1941) and MATHER, or to test »the criteria of scale» of the latter (MATHER, 1949).

II. VITALITY, HETEROSIS AND DOMINANCE.

The true significance of the terms »heterosis» and »vitality» or »vigor» is debated in genetical literature and an unequivocal definition of the latter hardly exists. The meaning of »vitality» is more or less dependent upon the point of view of the person using the term and from different view-points the same organism may be said to have a very high or a very low vitality (*e. g.* a vegetatively luxuriant but fully sterile species hybrid). The word is ambiguous and in cases where misunderstandings are possible it should be replaced by some adequate and definite term. In this paper »vitality» or »vigor» has been used only when the meaning is absolutely clear.

The use of the vague term »vitality» is probably to a great extent responsible for the confusion in the use of the term »heterosis» and the discussion of its meaning. The term, however, belongs to genetics and was introduced and defined by a geneticist (SHULL, 1914, 1948). SHULL writes: »To avoid the implication that all the genotypic differences which stimulate cell-division, growth, and other physiological activities of an organism, are Mendelian in their inheritance, and also to gain in brevity of expression, I suggest that instead of the phrases, 'stimulus of heterozygosis', 'heterozygotic stimulation', 'the stimulating effects of hybridity', 'stimulation due to differences in uniting gametes', etc., which have been used by myself and others, the word 'heterosis' be adopted». In a foot-note he adds: »The word 'heterozygosis' has been occasionally used for this 'stimulus of hybridity', but it should not be considered available for such restricted usage even by those who hold that all inheritance is essentially Mendelian, for the reason that, as used by its originator, Dr. Spillman, and also as rather generally used in genetic literature, the word 'heterozygosis' signifies only the state of being heterozygous». The definition is, perhaps, somewhat ambiguous, which may have contributed to the confusion in its use, and a more

definite and unequivocal definition would have been desirable. In a paper of 1948 SHULL contributes to a clarifying of the subject: »The word 'heterosis' ... should be *free from every hypothesis.* Doctor East and I used the word 'stimulation' and many others used the expression 'hybrid vigor' in discussing these phenomena, but both 'stimulation' and 'vigor' are ambiguous terms and refer solely to the fact that there is increased size or other valuable qualities in the cross-bred as compared with the pure biotypes or the different species or different varieties whose union produced the cross-bred in question». And, further: »By definition, heterosis is the *increase* of size, yield, vigor, etc. If there is no such *increase*, there is no heterosis».

WHALEY (*e. g.* 1944), POWERS (*e. g.* 1944), STERN (*e. g.* 1948), DOBZHANSKY (*e. g.* 1950), and others have created new »heterosis» concepts, each one more or less different from the others and from that of SHULL. In a series of papers DOBZHANSKY has criticized the terms heterosis and vigor as used by plant geneticists and breeders. Vitality or vigor may, as mentioned above, be taken to mean quite different things, and it is obvious that DOBZHANSKY in his studies on natural selection and evolution cannot accept their meaning as taken by, *e. g.*, plant breeders. In the opinion of the present author, however, it is not correct to use the term heterosis, once defined by another geneticist, to cover a different concept, give it a new definition and then criticize its use as originally defined. In a paper of 1950 DOBZHANSKY writes: »The advisability of applying the term 'heterosis' to cases in which heterozygotes are larger in body size, or show 'increase' in any 'traits', but no evidence of higher adaptive value compared to the corresponding homozygotes, is open to question. Perhaps the word 'luxuriance' would be a better designation for such cases, the word 'heterosis' or 'euheterosis' to be used for adaptive superiority of heterozygotes to homozygotes». It would seem to be better if DOBZHANSKY would coin a new term for his special form of »hybrid vigor», rather than use an old and more comprehensive one and compel its replacement by another.

GUSTAFSSON (1951) has tried to bring some order into chaos: »Heterosis evidently consists of three different part-processes; (1) What may be called *the somatic type of heterosis*; the vegetative system is affected (with or without competition). DOBZHANSKY (1950) designed this phenomenon as luxuriance. (2) There is *the reproductive type of heterosis*; the reproductive capacity (the number of pistils, ovules, pollen grains, seeds) is greatly influenced (with or without competition). And (3) there is *the adaptive type of heterosis*; a genotype puts

out a superior number of new individuals, which survive and increase in following generations under a continuous competition with other genotypes. It is to the third process DOBZHANSKY will restrict the use of the term heterosis».

It is obviously important to distinguish between the different phenomena but this necessity does not call for the use of »luxuriance» as a term for what SHULL defined as heterosis. The present paper deals with GUSTAFSSON's somatic type of heterosis, which in itself is a complex conception, including a large number of different phenomena, all of which have at least one thing in common, *i. e.* that F_1 in one way or the other is superior to the parents. In accordance with the opinion here expressed the author has preferred to use the term »heterosis» in discussing these phenomena.

POWERS (1944), STERN (1948), and HULL (1949) combine heterosis and dominance into one series; F_1 may be intermediate between the parents (no dominance), more similar to (partial dominance), or identical with (complete dominance) one of them or it may be superior to the superior of the parents or inferior to the inferior one in which case the authors use the terms heterosis and negative heterosis, respectively. This use of the term dominance can be correct only in monofactorial hybrids, in which case the terms superdominance (FISHER, quoted from NYBOM, 1950) and overdominance (HULL, 1949) are synonymous with heterosis or negative heterosis in the series mentioned. When introducing the term dominant, MENDEL used it to describe the relation between two alleles in one locus. The use of »dominance» in discussing quantitative characters, determined by polygenes, means the inclusion into the term not only of true dominance but also of different interactions between the genes. For the more complex phenomenon WIGAN (1944) has suggested the term »potence»: »... it is the potence which polygenic combinations show with respect to their homologues which is detected and measured in polygenic experiments and not the dominance or interactions of the individual polygenes». This distinction is important and must be remembered. MATHER (1946) in discussing the terms considers it to be permissible to use »dominance» for the more complex phenomenon.

Different authors have used different basic values in determining the »increase» in F_1 as compared with the parents. Several investigators (MÜNTZING, 1945; COFFMAN and WIEBE, 1930) have compared F_1 with the mean (arithmetic or geometric) of the parents, which may seem to be logical from the point of view of JONES' (1917) theory of linked.

more or less dominant quantitative genes as the cause of heterosis (VEATCH, 1930). On the other hand VEATCH (*l. c.*) writes: »... it was thought that comparisons made with the better parent would be of greater significance than those made with the average ...» and WHALEY (1944): »Superiority over the better parent may be the more valid measure» (compare ASHBY, 1937). With regard to SHULL's (1914) definition of heterosis, it seems to be more correct to compare F_1 with the superior parental line, and in regard to adaptive heterosis it seems evident that it can influence the final result of selection only if F_1 is superior to both parents. The present author has compared F_1 with the better parent except in some cases where a comparison has been made with the results of MÜNTZING, in which cases the average has been used as basic value.

III. MATERIAL.

The material for the present investigation has largely been obtained from MÜNTZING, who has kindly handed over samples of his pure lines of *Galeopsis*, for which the author wishes to tend his most sincere thanks. A few of the lines used have been collected by the author, but most of them were collected in 1922—1925 by MÜNTZING. They all belong to one of the four species *speciosa*, *pubescens*, *Tetrahit*, and *bifida* of the subgenus *Tetrahit* REICHB. MÜNTZING has described the lines, their genetical constitution and their cytology in a series of papers (MÜNTZING, 1927, 1929, 1930 a, 1938, 1945, *etc.*).

Galeopsis speciosa and *pubescens* are diploid with $2n = 16$; *Galeopsis Tetrahit* and *bifida* are tetraploid with $2n = 32$. All the lines studied are summer annual. The tetraploid species are more markedly autogamous than the diploid ones, which latter have much larger flowers and in their natural habitat more or less behave as insect pollinated allogamous plants. All the species are, however, completely self-fertile, and it has been possible to obtain and keep pure lines. As mentioned above, most of the lines used in this study were collected by MÜNTZING some 25 years ago and they have been grown under controlled conditions and selfed by means of bags for about 25 generations. The lines are fully uniform and the homozygosity seems to be complete. All F_1 plants of one combination are, therefore, isogenous, and all variation within an F_1 or a parental line is caused by environment (with the exception of possible mutations).

One exception from the uniformity of the pure lines has occurred in the material of the author. In the *Tetrahit* line T—B there occurred in

1949 a single plant with vigorous growth and with flowers considerably larger than in normal T—B, more similar to those of the diploid species. The plant was completely sterile, only a very few giant pollen grains were developed — all other pollen being shrivelled — and no viable seed was obtained. Unfortunately, no good fixations of the meiosis were obtained and the chromosome number was not determined. About 2000 plants of T—B have been studied and the frequency of aberrant plants is less than 1 $^{0}/_{00}$.

The different pure lines are denoted as by MÜNTZING; their origin and some data about their characters are given in the following list:

Galeopsis speciosa:

S—C from Cambridge, England
S—K » Karup, Skåne (Scania), Sweden
S—Kv » Kvickjock, Lappland, Sweden
S—Sv » Sweden, probably Skåne

Galeopsis pubescens:

P—B from Austria (Rosenbach)
P—C » » , Spital

Galeopsis bifida:

B—B	from	Bucharest, Rumania	late
B—C	»	Kivik, Skåne, Sweden	early, very low growth
B—D	»	Gothenburg, Sweden	early
B—Dr	»	Draget, Halland, Sweden	intermediate
B—E	»	Gyllebo, Skåne, Sweden	late, low growth
B—F	»	» » »	intermediate
B—K	»	Karungi, Norrbotten, Sweden	very early, small, low and weak
B—R	»	Rumania	very late, tall, rich branching
B—S	»	Siberia	intermediate
B—H	»	Drakabygget, Halland, Sweden	early

Galeopsis Tetrahit:

T—B	from	Rörum, Skåne, Sweden	late
T—C	»	Riga, Latvia	early, tall for its earliness
T—D	»	Kivik, Skåne, Sweden	late, tall
T—F	»	Andrarum, Skåne, Sweden	intermediate
T—G	»	» » »	very late, tall, weak branching
T—I	»	Gothenburg, Sweden	intermediate
T—J	»	Andrarum, Skåne, Sweden	intermediate, low growth, short internodes.
T—X	»	Hörröd, Skåne, Sweden	early, low growth

A list of Swedish plants (HYLANDER, 1941) includes the three species *bifida*, *Tetrahit* and *speciosa* but not *pubescens*, and a phytogeo-

graphical atlas by HULTÉN (1950) gives the three former but not the latter species as growing in Sweden. They are probably introduced fairly recently, appear mainly in habitats influenced by cultivation and it is somewhat hazardous to use the material for evolutionary deductions.

IV. METHODS.

In order to obtain fully comparable material of parents and F_1:s artificial crosses have been made not only between the lines but also within them. It is important that the biotypes to be compared develop under equal conditions from the very first stages of the ontogenesis, thus even during the development of the embryo until the ripening of the seed. For crossing, buds have been emasculated in the afternoon, enclosed in paper bags and pollinated the next morning. The setting of seed has, as a rule, been quite good with the exception of certain species crosses; crosses between diploids and tetraploids have given no seed.

The seeds have been stored over winter in a cool attic; after weighing, they have been placed in moist filter paper on about April 1st. After two days, when the swelling of the seed had started, the embryos were liberated, freed from seed coat. In this way it was possible to obtain rapid and simultaneous germination, which would have been impossible without the operation mentioned, since whole seeds show a very wide variation in rapidity of germination, within the biotypes as well as, to a much higher degree, between them. The young seedlings grew at first on moist filter paper under glass bells on a germination thermostat of the type commonly used in seed testing work, during which time the growth of hypocotyle and radicula was measured. After about one week they were transplanted in soil in wooden boxes in a cold bed, at first under glass, later open. During this period measurements of leaves, height of plant and length of internodes were made. Finally, towards the end of May, the plants were moved to the field. The biotypes to be directly compared have always been handled simultaneously, and great care has been taken to obtain uniform treatment: the young plants have been placed in the boxes according to the plan for field planting, described below.

In 1945 only three different combinations were studied and it was possible to place plants of the biotypes to be compared in one and the same row. If the biotypes of the combination T—B × T—C are denoted: T—B = *, T—C = 0 and F_1 = +, the planting was made according to the following scheme:

* + 0 * + 0 * + 0
+ 0 * + 0 * + 0 * *etc.*
0 * + 0 * + 0 * +

In the following years the material was more extensive and it was impossible to use this or a similar plan. Each row was planted with five plants of one biotype, and the biotypes were grouped so that F_1:s with parents in common came in the same group with these. If a group contained, *e. g.*, seven biotypes, the rows were combined in blocks of seven rows, and within the blocks the different biotypes were placed in the order they would have had in the different rows of a 7×7 Latin square, *e. g.*, 1—7, 4—3, 6—5, 2—1, 5—4, 7—6, 3—2, 1—7, *etc.* The number of replicates was not, however, always a multiple of the number of biotypes in a group.

The distance between the rows was 60 cm with the plants 60 cm apart in the row, these distances having been determined in a preliminary test to be described later on in this section.

The height of the plants was determined at reasonably equal intervals, about once a week during the vegetation period, and in this way a curve for the growth in height has been obtained for each biotype. The date for the appearance of the first flower on each plant was noted. When the growth in height had ended and the plants began to ripen they were cut at the soil surface and weighed before and after drying: fresh weight and dry weight, respectively.

These weights, however, are not correct, since the shedding of ripe seeds cannot be avoided, whereby the plants lose more or less in weight. The loss by shedding is probably percentually lower in plants with poor seed setting, *e. g.*, the partially sterile F_1 plants, than in fully fertile ones. It is difficult to estimate exactly the size of the loss, but a small experiment showed that T—B, which is completely fertile, lost about 25 % of its dry weight, whereas F_1 of the fairly sterile cross T—B $\times$ B—E (about 30 % good pollen) lost about 15 %.

Samples of pollen were taken in the morning from newly opened flowers. Fixation and staining have been made with Belling-glycerine and the percentage of good pollen determined in the usual manner. MÜNTZING has studied the relation between the percentage of good pollen, as determined in Belling preparations and the germination of the pollen. The percentage of germination was always lower than that of »good pollen» but the correlation was strong. He also found that »the more there is of morphologically good pollen, the more of this pollen is

also functional» (MÜNTZING, 1930 a). The seed setting has been determined on the ripe plants; when the seeds are shed it is easy to count the shrivelled seeds or undeveloped ovules. The seed setting is given as number of seeds per flower.

The statistical treatment of the data has on the whole followed the methods given by BONNIER and TEDIN (1940). In some studies of variability the coefficient of variation (JOHANNSEN, 1927; KEMP, 1942) has been discussed and specially developed methods used. According to the method suggested by BONNIER and TEDIN the significance of t-, v^2- and χ^2-values has been denoted by asterisks: $***=P<0{,}001$, $**=P<0{,}01$, $*=P<0{,}05$ and $0=P>0{,}05$. The same signs between two values in a table denote the degree of significance of the difference between the values.

In several cases in the present material it has been impossible to apply the methods of analysis of variance because of incomplete orthogonality. In such cases the »classical method» (JOHANNSEN, *l. c.*; BONNIER and TEDIN, *l. c.*) has been used. As an example it may be mentioned that a separation of the single degree of freedom for »difference between F_1 and mean of both parents» would require an equal number of individuals in all three biotypes.

As the experiments are planned and planted a complete analysis of variance should be possible. There are, however, always some individual plants missing, because of diseases, damage by hares or accidental maternal plants among the F_1. Altogether about 5 % of the plants are missing and the result is a non-orthogonality which makes a direct analysis impossible. It is possible to estimate values for the missing plants, but this involves laborious calculations and admits of a certain degree of uncertainty. The method has been applied on the material of 1948, in order to obtain a comparison between this method and the one actually used. In this latter the classical method has been applied, each plant being counted as a variate. Since the plants are grouped in rows of five this will give too high a number of degrees of freedom for the error, and thus tends to an overestimation of significance. The rows are distributed according to accepted rules, but with the method used the variation between blocks cannot be eliminated, which again tends to an underestimation of significance. It is impossible to estimate the relative importance of the factors tending in opposite directions, but a comparison on the material of 1948 between the two methods discussed gave no marked difference between them. Under such circumstances the simpler method has been considered possible to

use, remembering that caution is necessary in judging the value of results, the statistical significance of which is determined by a not quite adequate method.

The measurement of characters such as height, weight and others often shows more or less skew distributions, which may depend upon the nature of the action of the genes, physiological limits to certain phenotypic characters, or other more or less involved phenomena. Already GALTON (1889) was of the opinion that the effect of the factors influencing quantitative inheritance was not arithmetic but geometric. The idea has later been developed by several investigators studying the inheritance of quantitative characters (RASMUSSON, 1933; MATHER, 1941, *etc.*; POWERS, 1941). As mentioned in the introduction, the main purpose of this investigation has been to study the occurrence of heterosis. F_1 alone has been studied and it has, therefore, been impossible to estimate the proper scales to be eventually used instead of the direct metric ones. It is the conviction of the author that the results obtained and the conclusions drawn are nevertheless valid.

A preliminary experiment was made in 1945 in order to study certain details in the experimental technique. This experiment gave some results of more general interest and is therefore briefly described. The main problem was the question of the influence of the distance between plants when two biotypes are competing, and at the same time the border effect was studied. It is probable that a plant with poor or no seed setting goes on growing, which might cause a stronger heterosis in sterile hybrids. To study the effect of a decrease in seed setting all buds were removed from half of the number of plants in the experiment. There were, thus, four distinct differences, *viz.* (1) between the lines T—B and T—D; (2) between the planting distances 30×30 and 60×60 cm; (3) between border plants and plants with neighbours on all four sides; (4) between normal plants and plants with the buds removed.

The experiment consisted of two squares, one metre apart, of 10×10 plants, the planting distance in one of them being 30×30 cm, in the other 60×60 cm. Every other plant in both directions was T—B, the others T—D. In every other row the buds were removed from all ten plants during the 2—3 first weeks of the flowering period, while the rest of the plants were left unmolested.

Plant height, fresh weight and dry weight were determined and the results are summarized in Table 1. Two plants were missing, and the classical method was used in the statistical analysis; the significance of the differences is indicated as described earlier in this section. The plots with the two planting distances were separated, but the soil was quite even, and it seems safe to attribute to the treatment the differences found, it being quite improbable that differences of the magnitude observed are caused by soil differences.

The line T—D has the same plant height at both planting distances. At 60×60 cm T—B is significantly lower, but at 30×30 it reaches the same height as T—D. It seems fairly evident that the close stand forces the T—B plants to grow

TABLE 1. *Competition between two pure lines of Galeopsis Tetrahit at different planting distances. Explanation in text.*

Character studied	Plant height				Fresh weight				Dry weight			
	Planting dist.				Planting dist.				Planting dist.			
	30 cm		60 cm		30 cm		60 cm		30 cm		60 cm	
Variety, treatment or place												
Line T—B	89,5	100	84,6	100	114	100	236	100	23,7	100	58,5	100
	0		***		***		***		***		***	
Line T—D	89,6	100	90,6	107	173	152	349	148	37,7	159	77,0	132
Treated plants	87,3	95	85,1	95	158	122	298	108	32,6	113	68,4	103
	***		***		*		*		0		0	
Untreatad plants	91,7	100	89,7	100	130	100	275	100	29,0	100	66,4	100
Border plants	89,4	100	87,0	99	170	134	328	127	36,4	135	76,2	124
	0		0		***		***		***		***	
Other plants	89,6	100	87,8	100	126	100	258	100	27,0	100	61,3	100
Mean	89,5	0	87,6		144	***	286		30,8	***	67,4	

higher in order to obtain light. The increase in height is not correlated with an increase in weight; the figures — especially those for dry weight — would rather indicate that T—B weighs less in comparison with T—D in the dense stand, and thus is somewhat suppressed by the competition.

The plants with the buds removed are significantly lower than the untreated ones, which must depend upon some kind of injury by the treatment and anyway gives no indication that sterility should cause an increase in height. On the other hand, the weight-data give a certain indication of higher weight in the treated plants. This might depend upon a continued growth of branches of the third, fourth and higher orders. It must be remembered that *Galeopsis* has a type of growth, with the central axis ending in two flowers, which makes the observed type of the effect of sterility upon continued growth the one to be expected.

The border effect is marked at both planting distances but more strongly so in the more dense stand, which again is to be expected.

V. CROSSES WITHIN PURE LINES.

As already mentioned, crosses have been made within the pure lines in order to obtain seeds of these which have from the very first stages developed under conditions equal to those of the inter-line crosses. This has given the author the opportunity to compare the offspring from selfings and inter-plant crosses within a number of pure lines.

As a rule, the seed after artificial crossing is somewhat poorer in quality than that obtained after normal selfing, probably because of disturbances caused by manipulation and bag-isolation. The plants from selfings are somewhat better developed than those from crossing and the latter show some tendency to a reduced fertility, but the differ-

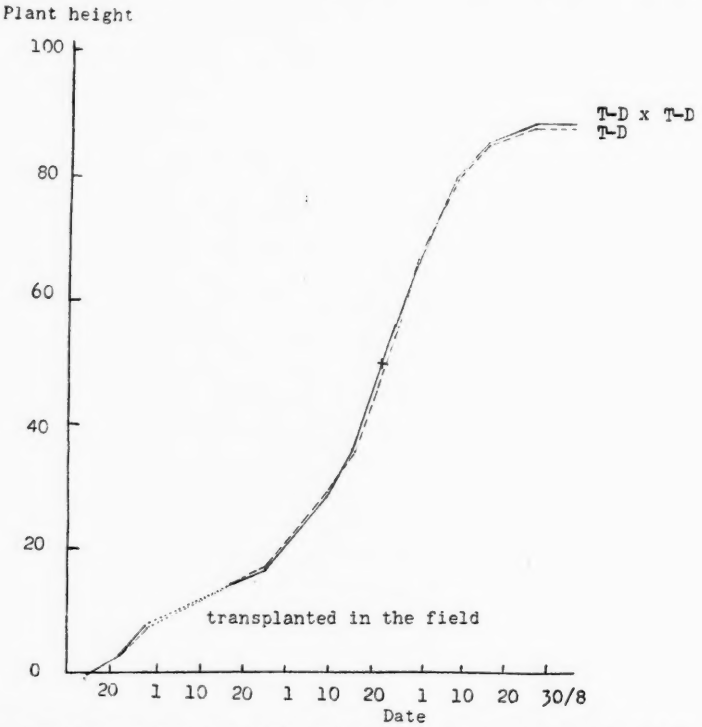

Fig. 1. Growth curves for plant height (cm) in 1946 for T—D (selfed) and T—D × T—D.

ences are insignificant. In most cases the agreement between the two groups of plants is good, as in the case of T—D compared with T—D × T—D, the growth curves of which practically coincide (Fig. 1). About the same agreement is found in the curves for T—B and T—B × T—B in Fig. 14, page 58.

VI. COMBINATIONS BETWEEN DIFFERENT LINES.

The eight pure lines of *Galeopsis Tetrahit* have been crossed in most of the possible combinations, the successful ones are tabulated in

	T—C	T—F	T—J	T—X	T—D	T—G	T—B	T—I
T—C	+	+	+		—	—	—	
T—F	+	+	+	+	+	+	—	—
T—J	+	+	+				—	
T—X		+		+	+	+	—	
T—D	—	+		+	+	+	—	—
T—G	—	+		+	+	+	—	
T—B	—	—	—	—	—	—	+	—
T—I		—			—		—	+

Fig. 2. Diagram of crosses studied. Between lines of *Tetrahit*. + = > 90 % good pollen, — = < 90 % good pollen; no sign indicates that the combination has not been studied.

Fig. 2. Within this species MÜNTZING (1938) has been able to group some of the lines into fertility-groups (*cf.* page 70), crosses between lines within a group giving fertile F_1:s, while crosses between lines from different groups give an F_1 with reduced fertility. T—B, T—D, T—G and T—I each belong to a separate group while the four remaining lines were considered to belong to one group (MÜNTZING, *l. c.*). Whether T—C should be included in this group will be discussed later on as well as the proper grouping of T—D and T—G.

Between nine of the ten *bifida* lines all possible combinations have been made and from most of the combinations F_1 plants have been obtained and studied as to pollen fertility. These studies also prove the existence in this species of sterility barriers. The results are shown in Fig. 3.

	B—H	B—B	B—C	B—Dr	B—E	B—F	B—S	B—R	B—D	B—K
B—H	+	—								
B—B	—	+	+	+	+	+	+	—	—	—
B—C		+	+	+	+	+	—	+	—	—
B—Dr		+	+	+	+	+	+	—	—	
B—E		+	+	+	+	+	+	+	—	—
B—F		+	+	+	+	+	+	—		
B—S		+	—	+	+	+	+	+	+	—
B—R		—	+	—	+	—	+	+	+	—
B—D		—	—	—	—		+	+	+	
B—K		—	—		—		—	—		+

Fig. 3. Diagram of crosses studied. Between lines of *bifida*. Explanation, see Fig. 2.

	T—C	T—F	T—J	T—X	T—D	T—G	T—B	T—I
B—B						—	—	
B—C		—	—	—				—
B—E	—	—			—	—	—	—
B—F	—	—			—	—	—	—
B—R					—	—	—	
B—K							—	

Fig. 4. Diagram of crosses studied. Species crosses between *Tetrahit* and *bifida*. Explanation, see Fig. 2.

	P—B	P—C	S—Sv	S—K	S—Kv	S—C
P—B	+	+	—			—
P—C	+	+	—			
S—Sv	—	—	+	+		—
S—K			+	+		—
S—Kv						—
S—C	—		—	—	—	+

Fig. 5. Diagram of crosses studied. Between lines of *speciosa* and *pubescens*. Explanation, see Fig. 2.

The two lines of *pubescens* have been crossed, as also the four lines of *speciosa*, but all of the latter combinations have not given seed. Within *speciosa* there are also sterility barriers, which are shown in Fig. 5.

A large number of crosses between the tetraploid species *Tetrahit* and *bifida* have been made, and the 22 successful combinations are tabulated in Fig. 4.

Crosses between *pubescens* and *speciosa* ($2n = 16$) are more difficult and at the best only a few seeds are obtained. Crosses between diploid and tetraploid species have failed (compare MÜNTZING, 1930 a).

The results with *Tetrahit* were mainly obtained during 1945—1948, but several combinations were studied in 1949 and 1950. The other species and the species crosses were studied mainly in 1949 and 1950, partly in 1947—1948.

VII. MAIN EXPERIMENTAL RESULTS.

1. SEED-SETTING, SEED-SIZE, GERMINATION.

Crosses having been made within and between lines it is possible to compare the seed-setting after inter-line pollination with the setting

after intra-line pollination. The crosses compared are made at one and the same time, since it might be of importance as to whether the crosses are made in the early part of the flowering period or considerably later.

TABLE 2. *Differences in seed-setting in per cent after use of alien pollen and pollen from the same line.*

Year	Difference	Stand. error	Number of combinations tested
1945	+ 3,632	± 2,970	19
1946	+ 3,945	± 2,239	21
1947	+ 5,988	± 3,530	13
Mean	+ 4,522	± 1,709	53
	t = 2,646*(*)	0,02 > P > 0,01	

Table 2 gives the differences in seed-setting in per cent between alien pollen and pollen from the same line, for crosses within *Tetrahit*. In all three years from which comparable material has been obtained the difference is positive; the mean value for all three years has a *P*-value between 0,01 and 0,02 which gives the difference a certain degree of significance.

In allogamous plants it is usual that alien pollen gives a better seed-setting than selfing. Among the numerous cases may be mentioned *Nicotiana*, where EAST and MANGELSDORF (1926) proved the existence of a system of self-sterility alleles, and the potato (HAGBERG and TEDIN, 1951) where no definite self-sterility occurs. It is somewhat more astonishing that a similar phenomenon can be observed in normally self-fertilizing species such as *Galeopsis Tetrahit* and barley (HAGBERG, unpublished). A possible explanation is that the superiority of alien pollen is a rudimentary survival from a period in the phylogeny of the species when they were still normally cross-pollinated. MÜNTZING (1930 b) has shown that *G. Tetrahit* is an allopolyploid, the result of an addition of the genomes or part of the genomes of *G. pubescens* and *speciosa*. These species are more allogamous, although not self-sterile. Possibly these species may have a system of alleles similar to that in *Nicotiana*, which has a weaker effect than in this genus but favours cross-pollination. In the allopolyploid *G. Tetrahit* this system still exists but its effect is reduced, causing the slight superiority of the alien pollen as regards seed-setting. No attempt has been made to penetrate further into this problem.

TABLE 3. *Weight of 1000 seeds of crosses within and between pure lines of Tetrahit and bifida.*

Combination	1945 F_1—P	1946 F_1—P	1947 F_1—P
T—B × T—B	4,2 g	4,6 g	3,2 g
× T—C	+ 0,5		
× T—F	+ 0,4		+ 0,8
× T—X	+ 0,2		
× B—E		+ 0,6	+ 0,7
× B—F		+ 0,2	+ 0,8
T—C × T—C	3,7 g	5,2 g	4,5 g
× T—B	+ 0,4	— 0,3	+ 0,8
× T—D	— 0,3		
× T—F	— 0,9	— 0,1	+ 0,1
T—D × T—D	3,7 g	4,4 g	3,9 g
× T—B		— 0,3	+ 0,4
× T—C	— 0,7	— 0,1	+ 0,7
× T—F	— 0,8	— 0,3	— 0,4
T—F × T—F	3,9 g	3,6 g	4,0 g
× T—B	+ 0,2	+ 0,8	+ 0,2
× T—C	— 1,2	+ 0,2	
× T—D	+ 0,7	± 0,0	
× T—I	+ 0,5	+ 0,9	
× T—J	+ 0,5	+ 0,3	
× T—X	— 0,4	+ 0,4	
T—F × T—F	3,9 g	3,6 g	4,0 g
× B—E		+ 0,2	— 0,5
× B—F		+ 0,4	— 0,3
T—I × T—I	5,8 g	6,5 g	5,8 g
× T—B		— 0,1	— 0,2
× T—C			— 0,9
× T—F	+ 0,1	— 0,3	— 0,6
T—J × T—J	4,4 g	4,6 g	3,2 g
× T—B	— 0,7	+ 0,1	+ 2,1
× T—F	— 0,2	— 0,1	+ 1,1
T—X × T—X	4,1 g	5,2 g	4,4 g
× T—B	— 0,4	+ 0,2	— 0,1
× T—F	+ 0,3	+ 0,1	+ 1,0
B—E × B—E		5,3 g	4,4 g
× B—F		— 0,5	— 0,3
× T—B			+ 0,4
× T—F			+ 0,3
B—F × B—F		5,1 g	5,4 g
× B—E			— 0,6
× T—B			— 1,3
× T—F			+ 0,4

The seeds obtained after crossing have been weighed and a comparison between the weights of seeds in intra- and inter-line crosses is given in Table 3. In 37 cases the seed after inter-line crossing is heavier than after intra-line pollination, in 27 cases there is a difference in the opposite direction, one case shows no difference at all. ASHBY (1930, *etc.*) is of the opinion that heterosis is caused by a superiority for F_1 already in the embryonic stages and that, therefore, heterosis in weight of embryo or seed is correlated with, and thus a measure of, the degree of heterosis of the full-grown plant. The correlation diagram in Fig. 6, including the data from 1945—1947 for crosses within *Tetrahit* and *bifida* and between the species shows no such relationship between the embryonic heterosis and that of the ripe plant. The species hybrids show a very marked heterosis in the full grown plants and they also usually have large seeds, but in one of the combinations the seed is rather poorly developed, while the heterosis in dry weight of the ripe plant is very strong. In some combinations the heterosis is very marked

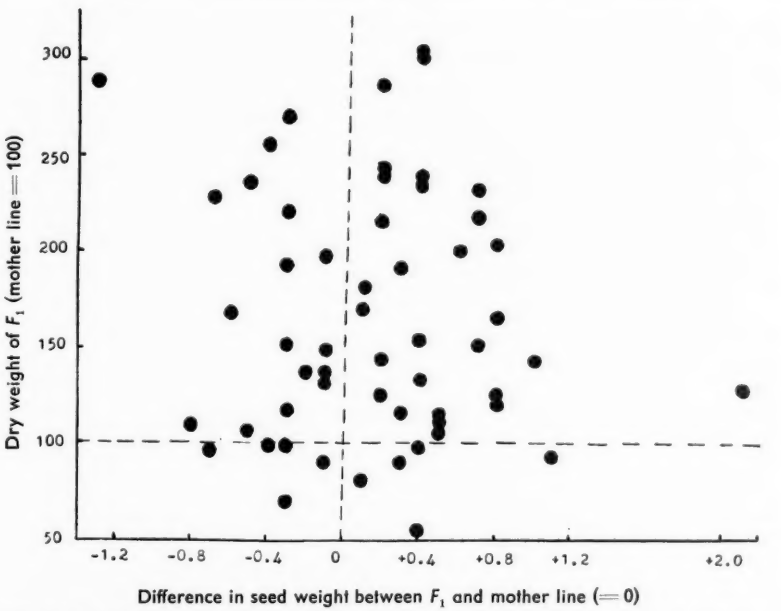

Fig. 6. Relation between superiority in seed-weight and the final degree of heterosis, estimated as the dry weight of F_1 in relation to the mother line.

already in the embryo, but the »final» heterosis is not conditioned only by the superiority of a larger seed but by several other factors, acting in later stages of the ontogenesis.

As a rule, the germination, after the embryo has been freed from seed coat, has been rapid and even. In some cases, especially when T—F has served as mother, the germination has been weak because of some kind of seed-rot. The seeds of T—B usually show a somewhat delayed germination, just as the development of the seedling of this line is rather slow; it is also one of the latest in the material. Seeds of F_1 combinations with T—B as mother line and a *bifida* line as father (B—B, B—E, B—F and B—R investigated) show a distinctly inhibited germination. The embryos may lie on filter paper under glass bells for 2—3 months without showing any tendency to growth. Then suddenly one or a few start growing, to be followed after a couple of weeks by some more. Fig. 8 shows a photo of embryos after one month on the germination thermostat; below there are a few which have just started growth. The curves in Fig. 7 show the percentages of germinated em-

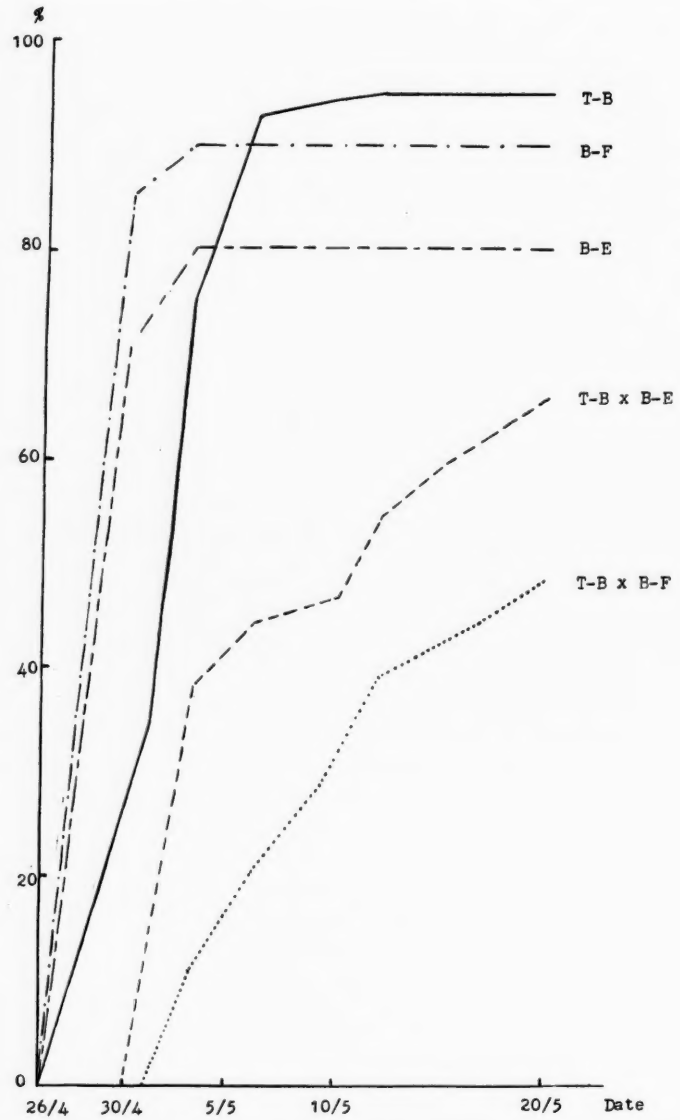

Fig. 7. Percentages at different times germinated embryos of the crosses T—B × B—E and T—B × B—F and the parental lines (1947).

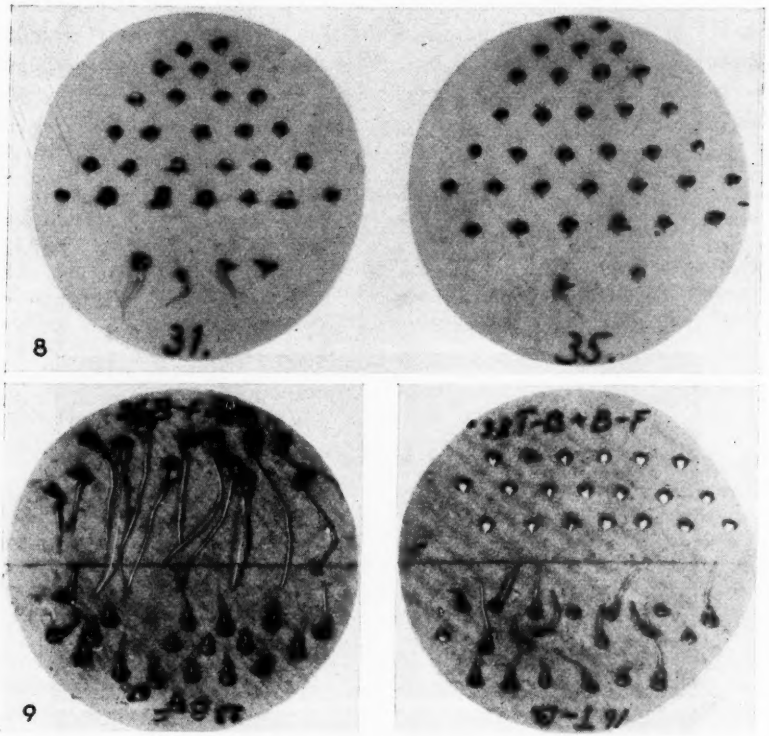

Fig. 8. Embryos of the crosses T—B × B—E (31) and T—B × B—F (35), one month after removal of seed coat and endosperm (1947). — Fig. 9. Embryos and seedlings of the cross T—B × B—F. Differences between reciprocal crosses and heterosis in the growth of the radicula.

bryos at different intervals for the crosses T—B × B—E, T—B × B—F and the parental lines. B—E and B—F germinate normally, rapidly and evenly, the germination of T—B is somewhat delayed (about 3 days) and somewhat uneven, F_1 is still more delayed and uneven, after about one month not 50 % of the embryos of T—B × B—F have germinated. The cotyledons of these embryos are at this stage swollen and fully green but a microscopic study of the radicula shows the cells filled with drops of oil, resting nuclei and no cell-divisions.

As shown by Figs. 9 and 10, the reciprocal crosses have a markedly different behaviour. If the *bifida* lines are used as mothers the seed germinates normally with rapidly growing radicula, in both cases more

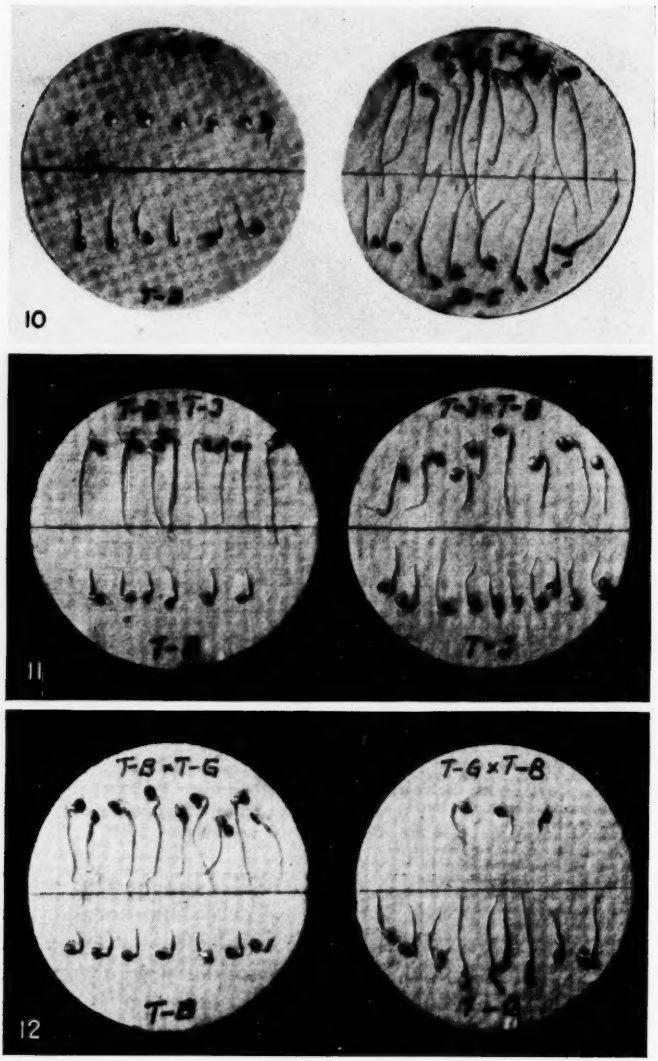

Fig. 10. Embryos and seedlings of the cross T—B × B—E. Differences between reciprocal crosses and heterosis in the growth of the radicula. — Figs. 11 and 12. Seedlings of the crosses T—B × T—I and T—B × T—G. No differences between reciprocals, marked heterosis.

rapidly than in the seedlings of the most rapidly growing parental line. This difference between the reciprocals has been obtained in all the combinations studied between T—B and *bifida* lines. In combinations between T—B and other *Tetrahit* lines there is no similar difference between the reciprocals, as illustrated by Figs. 11 and 12, where T—B × T—I and T—B × T—G show a rapid and even germination.

The inhibition of the germination in species hybrids with T—B as mother line may be caused by: (1) the lack of some substance, necessary for the cell division; (2) the existence in too high a concentration of some substance (possibly an enzyme) which inhibits germination; (3) the permeability is too low, the waxen cover of the embryo is too thick. The last explanation is hardly probable, since the swelling of the embryos is normal and chlorophyll develops, only the cell divisions are failing.

The seeds have been treated by temperature shocks — heat and cold — and the glass bells on the germination thermostat have been darkened, but without result. Nor were any results obtained by treating the embryos with heteroauxin in concentrations of 1 or 10 p. p. m.

Some of the substances within the vitamin B complex as well as some other substances were tested in 1948. In one experiment 16 seeds of the F_1 T—B × B—E were steeped for 7 hours in water or water solutions of the substances listed below and after 3 days the numbers of germinated seeds were counted and the length of the radicula measured, if of 1 mm or more. The results were as follows:

Substance	Conc.	Number germinated	Length of radicula
Pyridoxinhydrochloride (B_6)	0,01 %	13	40, 39, 22, 10, 9, 9, 1
Aneurin	0,01 »	6	4, 3, 1
Lactoflavin	0,001 »	5	26, 21, 10, 2
Nicotinic acid	0,01 »	5	4
Nutrient solution (Knop)		11	35, 29, 17, 7, 7
Tap water		9	40, 30, 22, 6, 1, 1
Distilled water		8	14
No treatment		0	

In another experiment 19 seeds of the cross T—B × B—F were steeped in a 0,01 % solution of pyridoxinhydrochloride for 14 hours, 15 seeds for the same time in tap water, while the remaining 17 seeds were left on filter paper under the glass bell. Result:

Treatment	Number of seeds treated	Number of seeds germinated after 3 days	7 days
B_6, 14 hours	19	6	13
Tap water, 14 hours	15	2	8
No treatment	17	0	1

A χ^2-analysis shows no significant difference between tap water and B_6, but a high significance ($\chi^2 = 14{,}4^{***}$) for the effect of steeping.

In seed testing a steeping in a 0,75 % solution of H_2O_2 is used to stimulate the germination of slowly germinating seed lots of cereals (KIESSLING, 1911, quoted from MAC KEY, 1951). This method was tested on 30 seeds of the cross T—B × B—R, one third of the lot being steeped in H_2O_2, another in tap water and the third one left untreated. H_2O_2 showed good effect with 100 % germination, but the seeds were damaged and the embryos weak. A somewhat lower concentration of peroxide might give a better result. The seeds steeped in tap water germinated most rapidly but more unevenly, probably depending upon different concentrations in different seeds of an inhibiting substance.

The experimental material is rather small but it shows fairly clearly that the cause of the inhibition of germination is the presence of some inhibiting substance, while a further stimulating effect of B_6 is not improbable (*cf.* ROBBINS and SCHMIDT, 1939). The results here related allow, perhaps, the conclusion that a substance, inhibiting germination, is produced in excess by a *bifida* genome plus a *Tetrahit* genome, when working in the plasma of T—B.

In this connection it may be pointed out that when the embryos have once started to germinate they show the rhythm of growth characteristic of the reciprocal combination and if they germinate early enough to reach full ripening in time they also show a marked heterosis. Due to the delayed germination there are, however, usually significant differences between the reciprocals in plant height and plant weight.

2. OBSERVATIONS ON SEEDLINGS.

A number of different observations, measurements and weighings have been made on the young seedlings and during their further development while planted in boxes. The primary data are available at the Institute of Genetics, Branch Station, Svalöf; only a summary of the results is given here.

The weight of the embryos was determined in 1945 two and three days after the liberation. As regards seed-weight, F_1 was on the whole equal to the parents without significant heterosis.

The length of the radicula was measured at different intervals. Already at this stage the species crosses, as a rule, showed a more or less increased rate of growth in comparison with the parental lines. Two examples may be given as illustrations. In 1948 20 seedlings of each biotype in the combination T—B × B—F were treated exactly alike in order to obtain material for the fixation of root-tips (*cf.* page 51). Five days after the liberation of the embryos the radicula was measured and the following mean values were obtained: T—B × T—B 12 mm; T—B × B—F 0 mm (no embryo had started growth); B—F ×

TABLE 4. *Length of radicula of 9 days old embryos of the combination T—B × B—R. 1951.*

Combination	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45 cm	M ± m	
T—B × T—B	21	2	14	15		8	1										6,5	(9,4)
T—B × B—R	29			1		1												(10,0)
B—R × T—B_1				2	1		4	2	2	2	4	6	4	4	2	2	27,7	27,0 ± 1,221
B—R × T—B_2				2	1	3	2	5		3	2	2	6	5	2	1	26,3	
B—R × B—R	1	1	6	9	13	14	11	6	1								14,5 ± 0,639	

Difference B—R × T—B — B—R × B—R : 12,5 ± 1,407 D/m = 8,88***

T—B 32 mm; B—F × B—F 8 mm. The rapid growth of the radicula in F_1 is evident also from Fig. 9.

Results obtained in 1951 from the combination T—B × B—R are presented in Table 4. As already mentioned, the germination of T—B × B—R is inhibited and only two embryos had started growth after 9 days. The reciprocal cross gave rapidly growing seedlings with the radicula significantly longer than that of the most rapidly growing parent, B—R.

This material also includes a study of the importance of the time of making the cross. B—R × T—B_1 was made on August 5th, B—R × T—B_2 on August 11th. There is no significant difference between the two groups.

Not only the species crosses but also the intra-specific ones show an increase in the rapidity of the growth of the radicula as shown by Figs. 11 and 12 for the combinations T—B × T—I and T—B × T—G. By an accident one of the reciprocals of the latter combination gave only three weak seedlings.

The size — length and width — of the cotyledons and the three first pairs of ordinary leaves have been observed. In this case the marked heterosis found in the growth of the radicula is lacking. In most cases F_1 is equal to, or at most somewhat superior to, the parental line having the largest leaves at the time of observation. In regard to morphological characters, number of side-ribs in the leaf, and of teeth in the leaf-border, F_1 is intermediate, or equal to the parental line with the highest number.

Also, in regard to the growth in height in these early stages, F_1, as a rule, follows the most rapidly growing of the parents. This is especially characteristic when one of the parents grows extremely rapidly, as T—F and to some extent T—C. Even if both parents grow fairly slowly at this stage, F_1 grows comparatively rapidly, however, which was

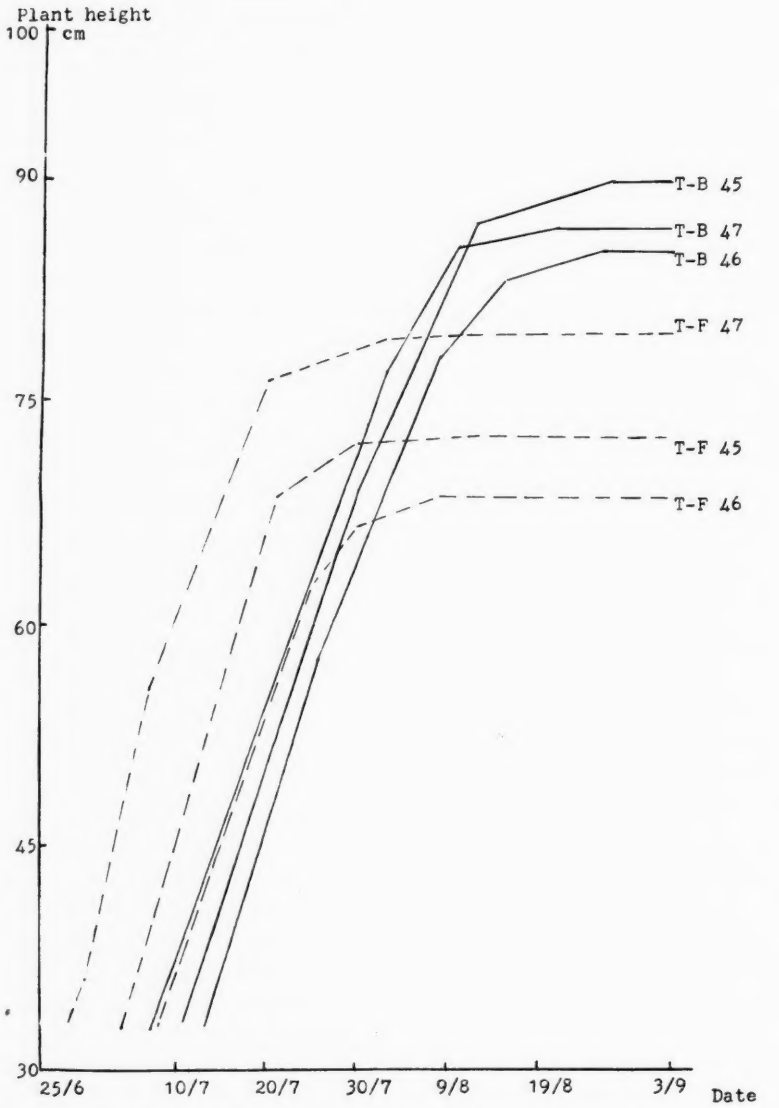

Fig. 13 *a*. Growth in height of some pure lines of *Galeopsis Tetrahit* in 1945—1947.

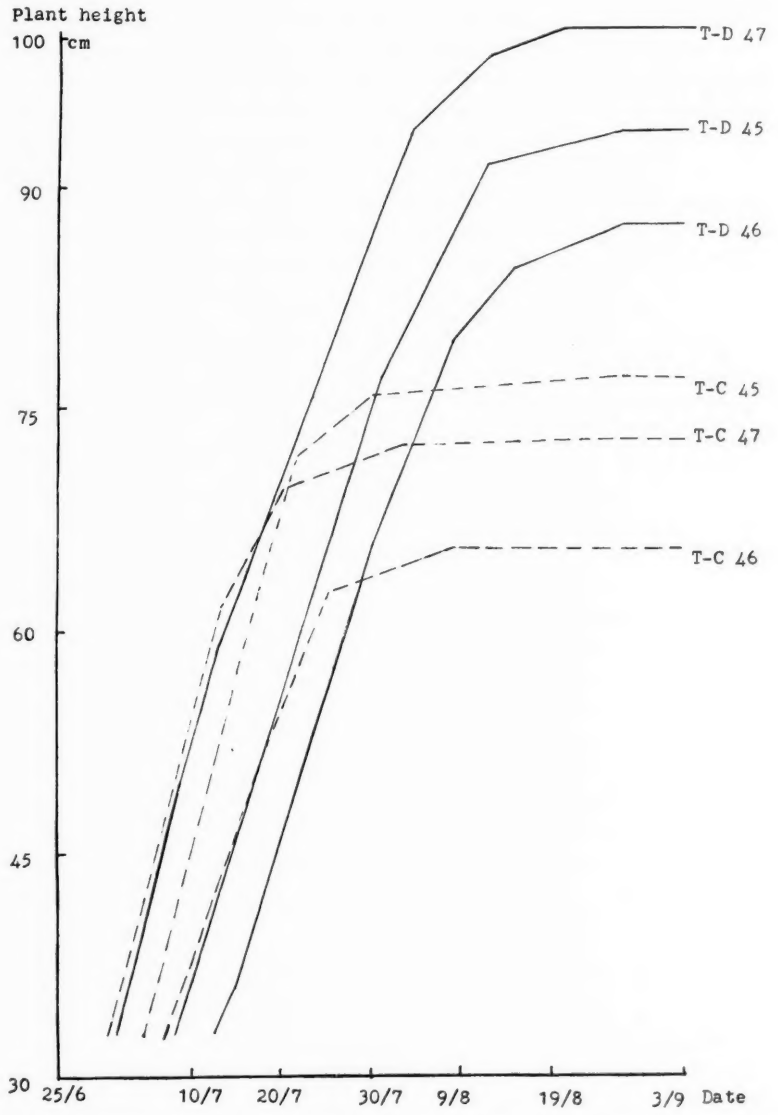

Fig. 13 *b*. Growth in height of some pure lines of *Galeopsis Tetrahit* in 1945—1947.

evident, *e. g.*, in the combination B—E × T—B. In most of the cases where F_1 at this stage is taller than the parents this superiority is caused only by a greater internode length. The number of nodes in F_1 in these cases is intermediate or equal to that of the parent with the highest number. In some of the species crosses, *e. g.*, T—B × B—R, with a very marked heterosis, F_1 is superior not only in internode length but also to a smaller extent in their number. Thus, cases are met with of heterosis in number as well as in size, although the latter type is by far the most common (compare MALINOWSKI, 1935).

3. GROWTH IN HEIGHT — GROWTH CURVES.

The plant height was determined once a week after the plants had been transplanted on to the field and until they were ripe. The planting in the field was carried out during the last week of May, ripening

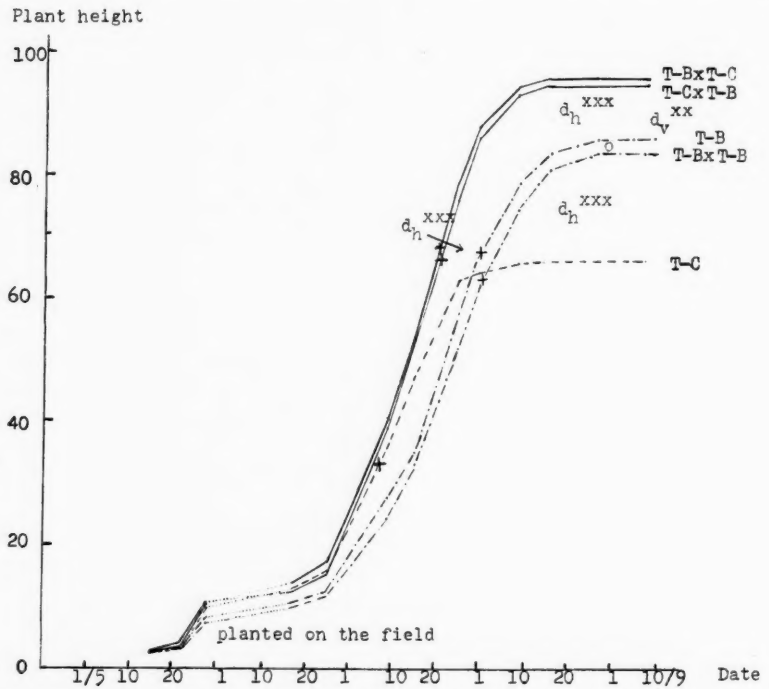

Fig. 14. Growth in height (cm) in the combination T—B × T—C. 1946. d_h***, d_v** *etc.* indicates the degree of significance of differences in height and weight at the time indicated.

occurring towards the end of August. No attempts have been made to determine the constants of the curves mathematically or to give coefficients of growth rate, but the curves obtained will be published directly in a diagram in Part II of the present paper. Numerical values for the growth rate would perhaps have been valuable but the diagrams offer a better comprehensive view of the material.

In order to demonstrate the influence of environment upon the growth curves Figs. 13 *a* and *b* show the growth curves for the four lines T—B, T—C, T—D and T—F in the years 1945, 1946 and 1947. All four lines have grown within the same small experimental garden in all three years and the differences are due mainly to variations in the climate, which varied very much between the years in regard to temperature, rainfall, hours of sunshine, *etc.* The different biotypes react distinctly differently to the changes in the weather conditions.

Here only a few of the growth curves will be discussed in greater detail. The general discussion will be carried out later on. T—B is a late line which grows vigorously during the later part of the vegetation period, flowers and ripens late, and which, therefore, in spite of its early slow growth, reaches a considerable height and weight. In combinations with early lines, *e. g.*, T—B × T—C, F_1 starts to grow rapidly as early as T—C, later on its growth curve is very similar to that of T—B, and this rapid growth goes on until the ripening, which occurs fairly late, although not quite as late as in T—B. The result is, as shown by the curves in Fig. 14, a marked heterosis.

The lines T—C and T—F are both comparatively early. F_1 shows transgression in earliness at the seedling stages of development and later on has the rapidity of growth of the most rapidly growing parent, which again results in heterosis. F_1 of the cross T—D × T—F shows a different »potence» in the different years when it has been studied. In 1945 it was as early as T—F, in 1946 and 1950 as late as T—D, and in 1947 and 1948 it was intermediate. In the combination T—F × T—I the result is the opposite to that found in T—B × T—C, F_1 showing the late start of T—I in combination with the early ripening of T—F, the result being that it is inferior to both parents.

T—I is comparatively tall but with fairly little branching. In height its combination with T—B is about intermediate, but there is heterosis in plant weight due to a combination of the richer branching of T—B with this intermediate height.

There are still greater differences in earliness and rate of growth between the *bifida* lines, which in many combinations give a marked

luxuriance as a result; this is not always, however, the case. Crosses with B—R, *e. g.*, rarely show any heterosis.

The species crosses *bifida* × *Tetrahit* in most cases give good examples of »hybrid luxuriance» (*cf.* MÜNTZING, 1930 a), but there are exceptions to the rule. Combinations where one of the parents is one of the tall vigorous strains T—G, T—D or B—R, rarely give an F_1 superior to themselves.

In the species hybrids between T—B and B—E or B—F heterosis becomes apparent in the early stages of the development of F_1, which shows transgression in earliness as well as in rate of growth and ripens as late as the latest of the parents. These F_1:s are fairly sterile, with only 30—40 % of good pollen and reduced seed-setting and it might be reasonable to suppose that the heterosis of the full-grown plants was the result of a continued vegetative growth, due to partial sterility. The growth curves, however, clearly show the existence of a marked heterosis already before flowering and also that the growth in height ends at the normal ripening time. Thus the sterile F_1 plants do not go on growing in height but it can be observed that the flowering continues and the plants keep green for rather long; probably they go on growing by branching longer than the fertile pure lines.

The F_1 of T—B × B—F grows more rapidly than T—B × B—E. Obviously B—F has a larger number of factors favouring growth than B—E, or, anyway, they are more effective when combined with the T—B genome. This difference between B—F and B—E is not equally marked in combinations with T—F. In these the early start of T—F is entirely »dominant» in both combinations, and the difference between T—F × B—F and T—F × B—E caused by the differences between the *bifida* genomes becomes apparent first in the flowering stage. In the cross between B—E and B—F there is only a weak and insignificant indication of heterosis.

4. PLANT HEIGHT AND PLANT WEIGHT AT RIPENING.

MÜNTZING (1945) is of the opinion that plant weight would be a better measure of »vitality» than plant height. He had only data for height available but considers it probable that height and weight are correlated. The question of such a correlation is of a certain interest for the present investigation and an analysis of covariance was made in 1945 on the material of the combination T—C × T—F. The regression is most probable not a straight line, but since both parent lines are of about the same height and the material falls within a fairly limited part

of the total variation curves for the species no special attempt has been made to study more than the simple straight line relationship. The correlation coefficients and their significance are as follows:

	T–C	F_1	T–F
r	+ 0,82	+ 0,79	+ 0,33
t^2	91,84***	61,21***	4,21*

Furthermore, the analysis of covariance shows: (1) The means of the biotypes deviate significantly ($v^2 = 15{,}22^{***}$) from the average intra-biotype regression line; (2) the intra-biotype regressions are perhaps different ($v^2 = 4{,}72^{*}$); (3) the deviations of the biotype means from the inter-biotype regression line are significantly larger than random deviations ($v^2 = 28{,}42^{***}$). The different regressions within and between biotypes are different and not parallel. There is clearly a correlation between height and weight of ripe plants within a pure line, but in studying inter-line crosses it is hardly advisable to draw conclusions as to weight from the height measurements.

Not even the weight, however, is an ideal measure of the total production of the plant. The fresh weight varies with the water content, and thus with the degree of ripening at harvest and the dry weight will cause an underestimation of the total production since most of the seeds shed successively after ripening. An attempt has been made (page 40) to estimate the loss by shedding. It is evident that it will lessen the weight in fully fertile crosses more than in partially sterile ones and, therefore, it is a contributing cause of the relation between the degree of heterosis and that of sterility observed by HAGBERG (1948). Besides the influence of shedding, caused by a definite weakness in the method used, the weight of more or less sterile F_1 plants is also influenced by the continued flowering and growth of small branches, observed in these. This error is shown, among other things, by the fact that dry weight in percentage of fresh weight is lower in sterile than in fertile plants.

Unfortunately, the crossing-work is rather tedious and the output is poor in comparison, *e. g.*, with maize. It has therefore been impossible to obtain sufficient material for a determination of the growth curves in regard to dry weight, curves which would have given an excellent measure of the vegetative productivity of different biotypes during different stages of the ontogenesis.

The results from some of the material of the years 1945—1950 are

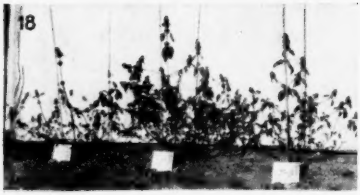

Fig. 15. T—F, T—F × B—E, B—E × T—F, B—E. One row of five plants of each biotype. 1948. — Fig. 16. T—B × B—F and the tallest of the parent lines, T—B. 1948. — Fig. 17. T—B, T—B × B—F, B—F, 1949. — Fig. 18. T—I, T—I × T—B, T—B, 1949. — Fig. 19. T—B, T—B × T—F, T—F, 1950. — Fig. 20. B—R, B—R × B—B, B—B, 1949. — Fig. 21. T—B, T—B × T—G, T—G, 1950.

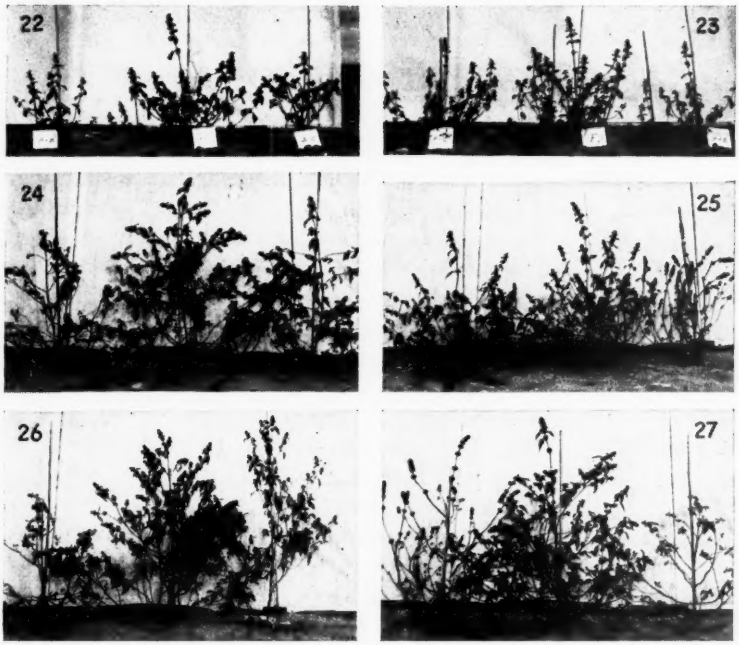

Fig. 22, P—B, P—B × S—C, S—C, 1949. — Fig. 23. P—C, P—C × P—B, P—B, 1949. — Fig. 24. B—E, B—E × T—B, T—B, 1950. — Fig. 25, P—B, P—B × P—C, P—C, 1950. — Fig. 26. B—E, B—E × T—G, T—G, 1950. — Fig. 27. T—I, T—I × B—E, B—E, 1950.

summarized in Table 5, which gives the relative values of height (H) and dry weight (W) at the time of ripening, the parent with the highest value being taken as 100. Where the number of individuals in an F_1 has been less than 10 the value is placed in brackets. Some of the F_1 combinations and their parental lines are shown in Figs. 15—27.

Root development. — A rapid development of the roots and a good competitive capacity is one of the conditions for a vigorous vegetative development of the plant. The competition in the soil is of great interest and should be studied in this connection (compare PAVLYSCHENKO and HARRINGTON, 1934).

Besides the measurements of the radicula, sporadic data about root development have been obtained. In one instance the three biotypes of T—B × B—R were grown in pots. At first F_1 behaved »normally» and grew more rapidly than both parents, but about the time of beginning florescence the difference began gradually to disappear, probably because of a crowding of the roots in the pots. On the first

TABLE 5. *Relative height and dry weight of some different F_1 combinations. 1945—1950. The best of the parents = 100. The figures in brackets are means from less than ten F_1 plants studied.*

Comb.	1945		1946		1947		1948		1949	1950	
	H.	W.	H.	W.	H.	W.	H.	W.	H.	H.	W.
T—B × T—C	112	142	109	117	103	128	106	128	(93)		
× T—D					103	143	107	153		106	129
× T—F			97	100	105	100	107	131	109	117	130
× T—G										92	122
× T—I					98	148			113	109	166
× T—J			100	150	93	163	87	123		103	148
× T—X			99	124	89	111	92	118		106	161
T—C × T—D			103	97	116	131	111	150		(99)	(83)
× T—F	113	134			105	97	113	111	(74)		
× T—G										96	95
× T—J									98		
T—D × T—F	103	139	98	109	98	99	100	133	96	(99)	(51)
× T—G										87	80
× T—I									100	115	140
× T—X										92	101
T—F × T—G										85	180
× T—I			91	76	88	70			91		
× T—J			93	108	96	90	98	94	108		
× T—X			86	100	85	65	89	97			
T—G × T—X										81	100
T—B × B—E					104	199	109	224	105	123	359
× B—F					123	239	115	211	112		
T—F × B—E					116	218	115	189		117	219
× B—F					111	217	118	253	110		
B—E × B—F					103	106	107	116		127	174

of July the F_1 plants had an average height of 33 cm, those of the tallest P-line only 27 cm, while on August 6th the corresponding heights were 70 cm and 72 cm, respectively. In this case T—B was the tallest of the parents, whereas B—R is taller when grown in the field. The roots were dried and weighed as also the plant above the soil-surface. The values were as follows:

	T—B	F_1	B—R
Plant height	72	70	65
Plant weight	10	15	11
Root weight	10	9	8

At the same time material of T—B and T—B × B—R was growing in wooden boxes, in one of them in a dense stand, in the other in a more open stand. In both boxes the more rapidly growing F_1 suppressed T—B. The root weight of the latter

in the box with the more open stand was 2,5 grams, in the other box only 1,5 grams, while the root-weight of the F_1 plants was on an average for both boxes 29,2 grams.

5. THE TIME FOR THE BEGINNING OF FLOWERING.

The growth curves clearly show that F_1 often combines the early start of the earlier of the parents with the more rapid growth of the later parent at the inflexion point of the curve, *i. e.*, at the time of most rapid growth. Thus F_1, as a rule, reaches the same maximum of growth rate as the parent superior in this respect; in some cases, especially among species hybrids, F_1 is superior to both parents in this respect. The question then arises as to whether the life rhythm of F_1 is more rapid in general. The time of beginning florescence is the only unequivocally defined biological phenomenon occurring during the period of most rapid growth.

The start of the florescence, *i. e.*, the date of the occurrence of the first opened flower on a plant, has been noted. Some data from these observations are given in Table 6. As zero date June 30th has been used. The differences in this case are given as the value of the date of F_1, less the average of the dates of the parents. In the calculations two decimals have been used, and to save space only the integers are given in the table. The significance of the differences was determined in 1945—1947, but not in the last three years. The relation of one and the same F_1 to its parents remains fairly constant from year to year. Thus during five years T—B × T—F has been 2—4 days earlier than the average of the parents, T—C × T—D 4—5 days later, T—D × T—F about 3 days later and T—B × B—E earlier than the average of the parents.

In several crosses (most of them not listed in Table 6) between late parents, F_1 flowers earlier than both parents, while in crosses between very early lines F_1 is often later than both parents. The question arises whether the variation between F_1:s is less than that between the averages of the corresponding P-lines. In 1950 65 different combinations were studied in this respect, the results being given in Table 7. The flowering date of each F_1 is a variate in the F_1 series, the average date of the corresponding parents being a variate in the P_m-series.

There is a general tendency for the F_1 series to lack the extreme values of the P_m-series and thus for F_1 to show a lower variability between biotypes than P. The quotients of variance of the three comparisons within a species or F_1 show a very low degree of significance, but the tendency is the same in all three cases and a summation of the data seems to be permissible. The result is a quotient of 1,84 for 62

TABLE 6. *Date for the beginning of the florescence in some different combinations. 1945—1950.*

Comb.	P_1	P_2	F_1	Diff.	P_1	P_2	F_1	Diff.	P_1	P_2	F_1	Diff.	P_1	P_2	F_1	Diff.	P_1	P_2	F_1	Diff.
	1945				1946				1947				1948				1949				1950			
T—B × T—C	34	7	18	−2**	30	7	21	+2***	26	1	13	0	25	0	14	+1								
× T—D									25	18	25	+3***	27	23	26	+1					24	19	22	0
× T—F					36	14	22	−2***	26	4	12	−3***	25	3	10	−4	32	12	20	−2	24	5	12	−3
× T—G																					24	29	26	−1
× T—I									30	12	20	−1					32	14	22	0	24	9	11	−5
× T—J					31	11	22	+1**	30	9	22	+2**	31	13	26	+4					24	9	18	+2
× T—X					31	6	19	0	29	3	19	+4*	31	3	22	+5					24	−10	10	+3
T—C × T—D					7	23	20	+5***	1	18	14	+4***	2	23	18	+5					1	19	12	+2
× T—F	7	11	11	+1***					1	4	2	0	0	3	1	0	12	11	10	−1				
× T—G																					1	29	18	+3
× T—J																	11	14	11	−2				
T—D × T—F	23	11	18	+1***	23	14	21	+3***	18	5	13	+2***	23	8	19	+3	23	12	19	+2	19	5	19	+7
× T—G																					19	29	22	−2
× T—I																	23	14	21	+3	19	9	16	+1
× T—X																					19	−10	9	+4
T—F × T—G																					5	29	17	0
× T—I					14	19	20	+3**	7	12	11	+1					12	14	11	−2				
× T—J					11	13	14	+2***	7	9	9	0	12	13	13	+1	12	14	12	−1				
× T—X					17	8	11	−2***	7	3	5	0	12	3	6	−1								
T—G × T—X																					29	−10	13	+3
T—B × B—E									25	18	20	−2**	25	22	17	−6					24	20	18	−4
× B—F									25	7	19	+3**	25	16	18	−2								
T—F × B—E									4	18	10	−1*	4	22	10	−3					20	5	7	−6
× B—F									4	7	7	+1*	4	16	13	+3	12	14	13	+1				
B—E × B—F									18	7	12	−1	22	16	16	−3					20	4	8	−5

TABLE 7. *Distributions of dates for beginning florescence of F_1 and the mean of the corresponding parents. 1950. 0 = June 30th.*

	−16	−12	−8	−4	±0	+4	+8	+12	+16	+20	+24	+28	M
G. bifida:													
P_m	1	1		1 1	2 4	1 2	4 1	3 1	4 1	2 1	2		7,25
F_1			2		2 3	3 3	3 2	4 3	4 2	1			7,06
G. Tetrahit:													
P_m						1 1	2	1 3	3	1	1	1	14,57
F_1							1 3	2 1	2 2	1 1	1		15,64
G. bifida × *G. Tetrahit:*													
P_m	1			1 1	2	1	2 1	1 1	1 1	2	2 1	1	12,9
F_1				2	1 1	3 1	3	1 1	1 2	1	2		11,7

Analysis of variance:

	P_m	Sum of squares	Degrees of freedom	Variance		Quotient	P
1.	*G. bifida*	2880,00	31	92,90	rows	$^1/_5 : 1{,}81^*$	0,05 — 0,02
2.	*G. Tetrahit*	509,43	13	39,19	»	$^2/_6 : 1{,}58^\circ$	> 0,05
3.	*b.* × *T.*	2374,95	18	131,94	»	$^3/_7 : 1{,}95^\circ$	> 0,05
4.	Sum P_m	5764,38	62	92,97	»	$^4/_8 : 1{,}84^{**}$	0,01 — 0,001
	F_1						
5.	*G. bifida*	1591,88	31	51,35			
6.	*G. Tetrahit*	323,21	13	24,86			
7.	*b.* × *T.*	1217,68	18	67,65			
8.	Sum F_1	3132,77	62	50,53			

degrees of freedom, giving a value of P between 0,01 and 0,001, and the phenomenon may be said to be fairly definitely demonstrated. This result is further confirmed by the observations made in 1945—1949.

Another problem is whether F_1 and the parent lines react differently upon differences in annual climate. The answer was partly given in discussing Table 6: the relation between F_1 and the mean of the parent lines is about the same in all years. There is, anyway, no tendency for F_1 to become relatively early in late years or relatively late in early years, and this also holds good for the opposite phenomenon.

In the growth curves the date for the opening of the first flower has been marked with a + and the stage of development has been determined as the height at this date in per cent of the total height attained. There is a tendency for F_1 to start flowering at a later stage in the growth in height than that corresponding to the mean of the parents, but the variation between different combinations is consider-

TABLE 8. *Pollen fertility in all studied crosses between lines of Galeopsis. 1945—1950.*

	B—H	B—B	B—C	B—Dr	B—E	B—F	B—D	B—R	B—S	B—K
T—C					27	31				
T—F			57		48	49				
T—J			47							
T—X			36							
T—D					48	57		55		
T—G		29			26	38		40		
T—B		41			33	40		46		68
T—I			26		23	25				
B—H	*97*	86								
B—B	86	*95*	93	93	91	92	87	65	92	67
B—C		93	*96*	90	92	95	86	93	88	79
B—Dr		93	90	*96*	98	96	79	89	93	
B—E		91	92	98	*96*	93	88	91	92	48
B—F		92	95	96	93	*96*		89	97	
B—D		87	86	79	88		*89*	93	94	
B—R		65	93	89	91	89	93	*95*	94	53
B—S		92	88	93	92	97	94	94	*97*	64
B—K		67	79		48			53	64	*90*

	T—C	T—F	T—J	T—X	T—D	T—G	T—B	T—I
T—C	*93*	94	97		85	88	56	
T—F	94	*98*	96	97	94	93	68	69
T—J	97	96	*90*				76	
T—X		97		*96*	96	92	58	
T—D	85	94		96	*97*	91	86	76
T—G	88	93		92	91	*96*	79	
T—B	56	68	76	58	86	79	*95*	65
T—I		69			76		65	*97*

	P—B	P—C	S—Sv	S—K	S—Kv	S—C
P—B	*80*	94	25			4
P—C	94	*90*	30			
S—Sv	25	30	*97*	98		36
S—K			98	*98*		46
S—Kv						32
S—C	4		36	46	32	*97*

able and there is no statistical significance for the tendency mentioned. In four combinations the later of the parents shows full »potence» in this respect.

6. POLLEN FERTILITY AND SEED-SETTING.

As already mentioned, MÜNTZING (1929, 1930 a, *etc.*) in studying the pollen fertility in *Galeopsis* found that the pure lines have a very high degree of fertility, whereas partial sterility occurs in different F_1:s, even between pure lines within the species *Tetrahit*. He also grouped the lines into fertility groups as already described (MÜNTZING, 1938). A similar grouping is possible within *bifida*, although the reduction in fertility within this species, with a few exceptions, is not so strong

TABLE 9. *Pollen fertility 1945—1950 in F_1 of different combinations within Tetrahit and in some of the species hybrids. The figures in brackets give the number of plants studied.*

Combination	1945		1946		1947		1948		1949		1950	
T—B × T—C	51	(21)	57	(71)	58	(38)	56	(17)				
× T—D					77	(40)	88	(27)			90	(69)
× T—F			70	(96)	70	(59)	62	(52)	74	(19)	68	(29)
× T—G											79	(21)
× T—I					61	(43)			63	(24)	71	(44)
× T—J			76	(45)	78	(20)	75	(25)			78	(15)
× T—X			58	(71)	48	(11)	63	(22)			60	(27)
T—C × T—D			84	(37)	88	(40)	82	(29)			86	(5)
× T—F	95	(40)			95	(37)	91	(20)	94	(9)		
× T—G											88	(6)
× T—J									97	(21)		
T—D × T—F	97	(26)	89	(60)	96	(53)	98	(31)	95	(9)	98	(2)
× T—G											91	(14)
× T—I									78	(15)	75	(24)
× T—X											96	(31)
T—F × T—G											93	(12)
× T—I			69	(13)	70	(11)			68	(20)		
× T—J			90	(12)	97	(41)	97	(25)	97	(19)		
× T—X			97	(76)	97	(14)	96	(29)				
T—G × T—X											92	(20)
T—B × B—E					31	(37)	31	(86)	45	(14)	41	(14)
× B—F					40	(17)	38	(66)	45	(19)		
T—F × B—E					50	(36)	48	(112)			43	(30)
× B—F					48	(36)	46	(61)	62	(19)		
B—E × B—F					92	(45)	93	(58)			96	(8)

as in the crosses between *Tetrahit* lines. However, if a fertility of 90 % is taken as the border value between fertile and partially sterile the groups given in Fig. 3 are obtained. The corresponding fertility-figures are given in Table 8, these values being direct means for each biotype of the values obtained in the years it was studied. The annual variation in pollen fertility may be studied in Table 9, giving values for each year for the crosses within *Tetrahit* and for species crosses studied more than two years.

As seen from Fig. 2 and Table 8, the present author has made some small changes in MÜNTZING's grouping of the *Tetrahit* lines. T—F, T—J and T—X constitute one group. Closely related are the lines T—C, T—D and T—G, but the two latter of these lines, reacting more similarly, give partial sterility when combined with T—C. Within a group comprising the six lines mentioned four subgroups are thus distinguished. A similar grouping is observed within *bifida* where B—D, B—R and B—S form a subgroup of the large one, while B—K so far must be placed in a quite separate group.

As shown already by MÜNTZING (1930 a), the species hybrids *bifida* × *Tetrahit* are on the whole more sterile than the intra-specific crosses, but there is no discontinuity between the species barrier and the intra-specific sterility barriers. The fertility in the intra-specific crosses within *Tetrahit* as well as *bifida* varies from 48 % to 100 % of good pollen, that of the species hybrids from 23 % to 68 % good pollen.

The data in Table 10 derive from the material of 1947. It shows the occurrence of significant differences in pollen fertility between most of the possible combinations between on one hand T—F and T—B, on

TABLE 10. *Pollen fertility in crosses Tetrahit × bifida in 1947.*

Combinations	Percentage of good pollen													
	15	20	25	30	35	40	45	50	55	60	65	70	M ± m
T—B × B—E		2	8	8	8	7	3	1						$30{,}1 \pm 1{,}23$
T—B × B—F			1	1	3	4	2	4	2					$39{,}4 \pm 2{,}11$
T—F × B—E					1	5	5	7	9	6	2	1		$48{,}8 \pm 1{,}38$
T—F × B—F				1	2	3	7	8	8	7				$46{,}9 \pm 1{,}33$

Significance of differences:

T—B × B—E/T—B × B—F $t = 3{,}79^{***}$
T—B × B—E/T—F × B—E $t = 10{,}10^{***}$
T—B × B—E/T—F × B—F $t = 9{,}25^{***}$
T—B × B—F/T—F × B—E $t = 3{,}75^{***}$
T—B × B—F/T—F × B—F $t = 3{,}01^{**}$
T—F × B—E/T—F × B—F $t = 1{,}01^{\circ}$

TABLE 11. *Pollen fertility in crosses between different lines of Tetrahit and the bifida lines B—E and B—F. 1950.*

P_1/P_2	B—E	B—F	Diff.
T—F ×	47	49	+ 2
T—C ×	27	31	+ 4
T—B ×	32	40	+ 8
T—D ×	48	57	+ 9
T—I ×	23	25	+ 2
T—G ×	26	38	+ 12
Mean	33,83	40,00	+ 6,167 ± 1,68
		D/m = 3,67*(*)	0,02 > P > 0,01

the other B—E and B—F. That T—B and T—F give different results is, perhaps, not remarkable, since the hybrid between them shows a reduction of pollen fertility to about 65 %. It is rather striking, however, that there is a significant difference between T—B × B—E and T—B × B—F, in spite of the fact that the cross between B—E and B—F seems to be fully fertile. The difference between B—E and B—F in combination with *Tetrahit* becomes evident also in the material of 1950 (see Table 11) where combinations with the former show a distinct tendency to lower fertility than the corresponding combinations with B—F.

The conclusion must be that the »species-barrier» between *Tetrahit* and *bifida* is not uniform in its effect, but varies with the parent lines used, and also that there is no fundamental difference between the species barrier and intra-specific ones, but only a difference in degree (*cf.* MÜNTZING, 1930 a).

Also within the diploid species *pubescens* and *speciosa* there are sterility barriers, the intra-specific combination S—C × S—Sv showing only 36 % of good pollen (*cf.* MÜNTZING, 1938). This occurrence of sterility within the diploid species is an interesting phenomenon to which the author will return in the discussion of his results.

The seed-setting, which is a result of the fertility of the female gametes and the viability of the zygotes, is measured by the number of seeds per flower, as described in the section about methods. There is a close correlation between seed-setting and pollen fertility. In 1950 the coefficient of correlation between the two values for different biotypes was $r = + 0{,}922$ with a t-value of $27{,}04^{***}$. Under these conditions it will seem superfluous to give any detailed data about the seed fertility of the different biotypes.

7. SIZE OF CELLS.

In 1946 a series of measurements of the length of the stomata was made in the combination T—B × T—C which shows a marked heterosis. Samples of the epidermis were taken on a well defined place on

TABLE 12. *Length of stomata in the combination T—B × T—C. 1946. Units of measurement = units on the ocular micrometer used.*

Biotypes	n	M	M ± m	Difference $F_1 - P_m$	± m	t
T—B	60	8,02	8,162 ± 0,041			
T—B × T—B	68	8,29				
T—B × T—C	42	8,90	8,956 ± 0,044	+ 0,303	± 0,062	4,89***
T—C × T—B	30	9,03				
T—C	59	9,12	9,124 ± 0,077			

an equally well defined leaf from each plant and 15 stomata, selected at random, were measured on each sample. Table 12 gives the mean values for the different biotypes and combinations, and Table 13 the analysis of variance of the data. F_1 does not quite reach the value of

TABLE 13. *Analysis of variance of the data in Table 12.*

Cause of var.	Sum of squares	DF.	Var.	Rows	Quotients
1. Between biotypes	796,10	4	199,02	1/7:	70,20***
2. B + BB: others	734,32	1	734,32	2/7:	258,99***
3. B : BB	41,79	1	41,79	3/7:	14,74***
4. C : BC + CB	16,53	1	16,53	4/7:	5,83*
5. BC : CB	3,46	1	3,46	5/7:	1,22°
6. Within lines	2937,67	3880	0,76		
7. Between plants, tot.	720,16	254	2,84	7/13:	4,64***
8. » » B	129,88	59	2,20	8/14:	3,45*** 8 + 9/10 + 11: 1,30°
9. » » BB	183,56	67	2,74	9/15:	5,16*** 12/8 + 9: 1,89**
10. » » BC	95,06	41	2,32	10/16:	3,53*** 10/11: 1,72(*)
11. » » CB	39,18	29	1,35	11/17:	1,92**(*)
12. » » C	272,48	58	4,70	12/18:	7,85*** 12/10 + 11: 2,45***
13. Within » tot.	2217,50	3626	0,61		
14. » » B	536,37	840	0,64		
15. » » BB	505,37	952	0,53		
16. » » BC	385,90	588	0,66	16+17/14+ + 15 + 18:	1,152**
17. » » CB	295,40	420	0,70		
18. » » C	494,47	826	0,60		
19. Total	3733,76	3884			

TABLE 14. *Measurements of the diameter of pollen. 1945.*

Biotype	M	m	Biotype	M	m
T—B	11,17	± 0,087	T—C	11,44	± 0,101
T—B × T—C	10,93	± 0,144	T—C × T—F	11,10	± 0,156
T—C	11,33	± 0,100	T—F × T—C	11,13	± 0,147
			T—F	11,25	± 0,139

TABLE 15. *Analysis of variance of data concerning T—B × T—C in Table 14.*

Cause of var.	Sum of squares	DF.	Variance	Rows	Quotients
1. Between biotypes	203,69	2	101,84	1/5:	3,79°
2. B : C	29,26	1	29,26	2/5:	1,09°
3. F_1 : B + C	174,43	1	174,43	3/5:	6,50*
4. Within biotypes	3417,33	1887	1,81		
5. Between plants, tot.	1610,79	60	26,85	5/9:	27,15***
6. » » B	329,94	20	16,50	6/10:	17,90***
7. » » C	456,09	20	22,80	7/11:	22,42***
8. » » F_1	824,77	20	41,24	8/12:	40,12***
				8/6 + 7	2,10*
9. Within plants, tot.	1806,53	1827	0,99		
10. » » B	561,20	609	0,92		
11. » » C	619,40	609	1,02		
12. » » F_1	625,93	609	1,03	12/10 + 11	1,06°
13. Total	3621,01	1889			

TABLE 16. *Analysis of variance of data concerning T—C × T—F in Table 14.*

Cause of var.	Sum of squares	DF.	Variance	Rows	Quotients
1. Between biotypes	75,40	2	37,70	1/5:	1,97°
2. F : C	38,30	1	38,30	2/5:	2,00°
3. F_1 : C + F	37,09	1	37,09	3/5:	1,94°
4. Within biotypes	2006,81	987	2,03		
5. Between plants, tot.	1204,01	63	19,11	5/9:	22,00***
6. » » C	350,23	21	16,68	6/10:	15,79***
7. » » F	490,12	21	23,34	7/11:	37,39***
8. » » F_1	363,65	21	17,32	8/12:	18,70***
9. Within plants, tot.	802,80	924	0,87		
10. » » C	325,33	308	1,06		
11. » » F	192,27	308	0,62		
12. » » F_1	285,20	308	0,93		
13. Total	2082,20	989			

T—C but the difference is only faintly significant, whereas F_1 clearly has larger stomata than the average between the parents.

The variation between plants is significantly greater than the intra-plant variation in all biotypes, pure lines and F_1. The inter-plant variation in T—C is significantly greater than in F_1 or T—B, the latter two not showing any significant difference in this respect. It may be mentioned that T—C showed also in other respects a remarkably high degree of variability in 1946. The intra-plant variation is fairly significantly greater in F_1 than in any of the parent lines.

In 1945 measurements were taken of the diameter of 30 filled and »good» pollen grains per plant in the partially sterile combination T—B × T—C as well as in the fully fertile one T—C × T—F. The results are given in Table 14 and the analyses of variance in Tables 15—16. There are no significant differences between F_1 and the parents, but the means of the former are lower than those of the latter. The quotient $8/(6 + 7)$ in Table 15 indicates a somewhat greater inter-plant variability in F_1 than in the parents; there are no differences between the biotypes as regards intra-plant variability.

8. MUTABILITY.

MÜNTZING (1930 a, pp. 215—216) has described epidermis mutations occurring in F_1:s of *Galeopsis* hybrids. One mutation of this type has occurred and been studied in the present material. The mutant plants were periclinal and sectorial chimaeras in regard to the genes for flower colour. One branch or sector of the F_1 plant had red flowers, while the other flowers on the plant were white. The border-line between the two sectors was sharply defined; sometimes it passed through one branch which then carried red flowers in one sector, white ones in the other, the flowers on the border-line also showing this quite distinctly between one red and one white part of the corolla.

MÜNTZING (1930 a) has shown the existence in *G. Tetrahit* of one factor *R* for red flower colour and of another factor *H*, inhibiting the effect of *R* and giving dominant white. The white-flowering line T—B has the constitution *HHRR* and the red-flowering T—J is *hhRR*. F_1 is *HhRR* and just in the combination T—B × T—J the gene *H* seems to be especially unstable, since a higher frequency of mutant plants was observed in this combination than in the others with the constitution *Hh* (see Table 17). With the low frequency of mutants and considering the fact that the combination T—B × T—J has been chosen *a posteriori* from a statistical point of view, the significance should be judged very

TABLE 17. *Mutation frequency in T—B × T—J in comparison with other Hh biotypes. 1946 and 1948.*

Biotype	Normal plants	Plants with mutated sectors
T—B × T—J	81	9
Other *Hh*	908	11

$\chi^2 = 32{,}70$

After correction for continuity (FISHER and YATES, 1938): $\chi^2 = 28{,}32^{***}$

critically. As seen from Table 18, the mutants were fairly common in 1946 and 1948; in 1947 two cases were found and in 1949 and 1950 none. The table gives an analysis of the annual variation. Even in this case the significance must be judged very critically, and annual differences in mutation frequency are not definitely proved.

In 1948 one of the F_1 plants of T—B × T—J was a complicated chimaera with apparently no less than 3 different instances of mutation. A plant with more than one instance of mutation has also been found in the species hybrid T—B × B—E, in spite of the observation being complicated by the action of other genes and the incomplete epistasy of *H* in this case. In all other chimaeric plants probably only one mutation occurred.

As a rule, *H* is dominant in crosses within *Tetrahit*, but during one part of the summer of 1948 the dominance of *H* was not complete. From July 20th until about August 5th the flowers were light pink, while flowers opening on August 15th or later were pure white with the exception of mutated sectors. During the period of incomplete dominance it was possible to detect a mutation from *h* to *H* (*Hh* → *HH*), one plant having a sector (about $^1/_4$ of the whole plant) with just as pure white flowers as the homozygous *HH*.

In 1949 some plants of the combinations T—B × T—J and T—B × T—I were grown in greenhouses in a higher temperature than in the open field. Both F_1:s had pink to light red flowers, indicating the

TABLE 18. *Annual variation in mutation frequency.*

Year	Normal plants	Plants with mutated sectors
1946	328	12
1947	298	2
1948	661	8
1949	136	0

$\chi^2 = 13{,}17^{**}$; D.F. = 3; $0{,}01 > P > 0{,}001$

influence of temperature upon the dominance (compare *Primula sinensis*; KLEBS, quoted from BAUR, 1922).

In the species hybrids *Tetrahit* × *bifida* F_1 has more or less coloured flowers, and *H* is not epistatic over all the flower colour factors of *bifida*, at least not when heterozygous. The combination T—B × T—G in this respect behaves as a species hybrid.

All cases of mutations have been found in heterozygous plants. During the four years tabulated in Table 18 there were 22 observed cases among 1287 plants, giving a frequency of 1/58,5. Having in mind that in homozygous *HH* plants there are twice as many chances for a mutation from *HH* to *Hh* as from *Hh* to *hh* in the heterozygotes, the frequency of visible chimaeras among homozygous *HH* plants should be $2/(58{,}5)^2 = 1/1711$. A large number of *HH* plants have been studied by MÜNTZING and the present author in the course of the years, but no case of mutation has been observed in them. There is thus a fairly strong indication that the frequency of mutations is larger in the heterozygous than in the homozygous state. LAMPRECHT (1941) has found a higher degree of mutability in heterozygotes than in homozygotes and also a difference in this respect between different heterozygotes of *Pisum* (*cf.* STURTEVANT, 1939). GUSTAFSSON (1947) found a higher frequency of spontaneous mutations in new, cross-bred varieties with a certain amount of heterozygosity than in old »pure line» varieties of barley.

9. SUMMARY AND DISCUSSION OF DATA IN SECTIONS 1—8.

First of all it may be stated that F_1, as a rule, is »vital» and varies on the plus side of the average of the parents. The data here presented also give numerous instances of the occurrence of true heterosis (SHULL, 1948), or luxuriance (DOBZHANSKY, 1950), if these terms are taken to mean that F_1 is superior in vegetative characters to the best of the parent lines.

The superiority of the heterozygous (hybrid) genotype over the homozygous (pure) parent lines is often apparent at a very early stage in the ontogenesis. The percentage of seed-setting after pollination with alien pollen was shown to be higher than after the use of pollen from the same line. However, it must be remembered that the homozygous pure lines normally have a very good seed-setting after open pollination, better than what is obtained after emasculation and artificial pollination. It is quite possible that the unfavourable conditions resulting from the manipulation of the flowers in the latter case have a differential effect

upon homozygous and heterozygous embryos, thus causing the difference in seed-setting with different kinds of pollen. No study of the development of the embryo before the ripening of the seed has been carried out, however.

One observation may be mentioned in this connection: the disturbed germination of F_1 seeds of the species hybrid *Tetrahit* × *bifida*, when the *Tetrahit* line T—B is used as mother. The cause is probably the excessive production of some substance inhibiting germination, indicating a disturbance in the balance between the genotype of the embryo, representing a species hybrid, and the genotype of the mother plant.

The seed-weight and the size of the embryo are obviously of great importance in giving the seedlings a good start, and no doubt there are crosses where F_1 is superior already at this stage (*cf.* ASHBY, 1930, 1936, 1937 and LUCKWILL, 1937). These characters, however, are not decisive for the occurrence of heterosis, as shown here and in a number of other studies (EAST, 1936; SPRAGUE, 1936; LINDSTROM, 1935; HATCHER, 1940; WHALEY, 1944; and others).

In many F_1 combinations the young seedlings grow very rapidly in comparison with the parents (compare SPRAGUE, 1936), and several species hybrids already at this stage lay the foundation of their heterotic development. Other F_1 combinations, on the other hand, especially those between early and rapidly growing parents, are in the beginning intermediate or equal to the most rapidly growing parent in this respect.

The cell size in hybrids and their parents has been investigated, among others, by KOSTOFF and ARUTIUNOVA (1936), who studied cells in the peribleme in root-tips, in the palisade parenchyma of leaves and in the stomata. Their conclusion was: »Die Bastarde mit üppigem Wachstum zeigen nicht grössere Zellen als die Elternarten». Similar results have been obtained by MALINOWSKI (1935). SPRAGUE (1936) points out that heterosis is mainly caused by an increase in the frequency of cell divisions and, therefore, is mainly found in stages where growth is meristematic, not where it is caused by a stretching of the cells.

KOSTOFF *et al.* (*l. c.*) found that the cells of the stomata varied less in size than other cells and, therefore, were especially suitable for investigation. In the present material the cells of the stomata and the pollen grains are not larger in F_1 than in the parent lines.

Rapid development and competitive ability of the roots is a

necessary condition for a vigorous vegetative development of the plant and the competition in the soil is of great interest (PAVLYSCHENKO and HARRINGTON, 1934). The rapid growth of the radicula in certain F_1 combinations indicates that these F_1:s will obtain a clear advantage before the parents, and such an advantage is demonstrated in the combination B—R × T—B, where the F_1 plants in a dense stand practically completely suppressed T—B.

In most of the cases where heterosis has been apparent in the present material it has been manifest in the size and not in the number of the organs or parts, but some exceptions have also been observed. There are a few cases (page 58) with a heterosis in the number of internodes (compare MALINOWSKI, 1935). And in the more sterile F_1:s the growth continues by the formation of new branches of a higher order, and in these cases the *number* of branches increases in comparison with the parent lines. In many other plants, *e. g.*, as regards maize, heterosis has been manifest in size and not in number. In other plants (tomato, WHALEY, 1939; soybean, VEATCH, 1930) the size is changed only a little but the number increased. There is probably no fundamental difference between the two types of manifestation of heterosis, but their different prevalence in different organisms may be connected with the mode of growth, apically limited or unlimited.

The relations between the different biotypes vary to some extent from year to year, but in most combinations the relation between F_1 and the parents is fairly constant. Exceptions occur, however, *e. g.* in the combination T—D × T—F, which showed a marked heterosis in 1945 but behaved quite differently in 1946.

In a diagram in Part II (Fig. 34) the growth curves in height will be given for all the combinations studied. Further data are added, *viz.* pollen fertility, dry weight of the ripe F_1 plant in relation to the superior parent line, date of beginning florescence for F_1 and P. The largest number of combinations grown simultaneously was studied in 1950, and as a rule the data from this year have been used in the curves. If, however, the growth curves of 1950 for a certain combination deviate considerably from those from other years the values have been taken from a year which is most representative of the mean value of all years. The values in Fig. 34 are, thus, not always directly comparable; nor would this have been the case, however, if mean curves had been calculated, since the different combinations have been studied in different years. The method used to present the data seems to offer the best comprehensive view of the results.

In 51 of the 81 combinations studied F_1 is taller than the tallest of the parents, in 8 cases it is about equal to this, and in 22 combinations it is intermediate. In no case is F_1 equal to or lower than the lowest parent.

The dry weight of the plants presents a similar picture, the number of combinations which show heterosis is even larger in regard to this character, *viz.* 63. In one case, the combination T—F × T—I, the weight of F_1 is lower than that of both parents. The values are: T—F 76,4 g, F_1 66,1 g and T—I 94,6 g. The difference between T—F and F_1 is significant ($P < 0{,}01$). We should here have an example of what POWERS (1944) calls non-beneficial heterosis and STERN (1948) negative heterosis, which use of the term heterosis SHULL (1948), however, does not accept.

It is of interest to study the growth curves for the height in this case. The start of F_1 is about the same as that of the late parent T—I, and later on the growth is about intermediate. In height F_1 passes the earlier of the parents, T—F, but does not reach the height of T—I. On the other hand, the poor branching of T—I »dominates» over the rich branching of T—F, and thus F_1 does not attain the weight of the latter. A combination of several instances of unfavourable »dominance» or »potence» results in the inferiority of F_1.

An attempt at an analysis of the cases of heterosis in height shows quite the opposite conditions. Heterosis is found in 51 cases out of 81; F_1 often starts growing as early or earlier than the earliest parent and maintains this advantage until ripening. At the time of the most rapid growth F_1 again has reached the rapidity of the later parent, which usually at this stage is superior to the earlier one. The ripening, finally, is intermediate or more close to the latest of the parents.

The most striking cases of heterosis have been obtained in combinations between two very early lines. A couple of examples may illustrate this, *viz.* the species hybrid B—C × T—X and the intraspecific combination B—C × B—K. In the first one of these F_1 has a growth curve more similar to that of a medium early type, later in flowering and ripening than the latest of the parents. In flowering time, the character which can be fixed most exactly, F_1 is 10 days later than the latest parent. In contrast to the weak vegetative development of the parental lines F_1 reaches a fairly normal height and weight; the relative value of dry weight in comparison with the superior parent is 900.

The combination B—C × B—K shows a similar picture, but the difference between F_1 and the parents is not quite so marked as in the

species hybrid. To judge from the phenotypes the genotypic difference in regard to characters, varying parallelly within both species, is less between B—C and B—K than between B—C and T—X.

In crosses between two late lines, *e. g.* T—G and B—R, F_1 is usually earlier than both parents, in flowering as well as in ripening, the latter determined as the date when the growth in height is finished. The F_1 plants have the rapid growth of T—G but ripen earlier, and do not attain the height of T—G. They are higher than B—R, however, and show the rich branching of this parent. Their dry weight is high, 150 if the superior of the parents is 100.

A similar picture is shown by a number of inter- and intra-specific combinations between late lines: T—G × B—B, T—B × B—B, T—B × B—E, T—B × B—R, B—B × B—R, B—B × B—E, B—R × B—E and T—D × T—G.

Just as in the case of flowering time it seems as if the general growth rhythm of the hybrids between extreme parents tends towards an intermediate type.

(To be continued in the next issue.)

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ASYNDESIS IN MACROSPOROGENESIS OF DIPLOID, TRIPLOID AND TETRAPLOID CHRYSANTHEMUM CARINATUM

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AT the Institute of Plant Systematics and Genetics of the Royal Agricultural College of Uppsala there are plants of *Chrysanthemum carinatum* in cultivation. These have been grown from seeds which were procured in 1941 from the Seed Company at Weibullsholm; they are listed in the catalogue as number 924. After treatment with colchicine tetraploids were obtained, in the progeny of which tetraploids as well as some triploids occurred. The author's cytological studies of these three polyploid types reveal that there exist remarkable meiotical differences between macro- and microsporogenesis.

Diploid Chrysanthemum carinatum. — Diploid *Chr. carinatum* has the chromosome number $2n = 18$, as may be seen in a root tip mitosis in Fig. 1.

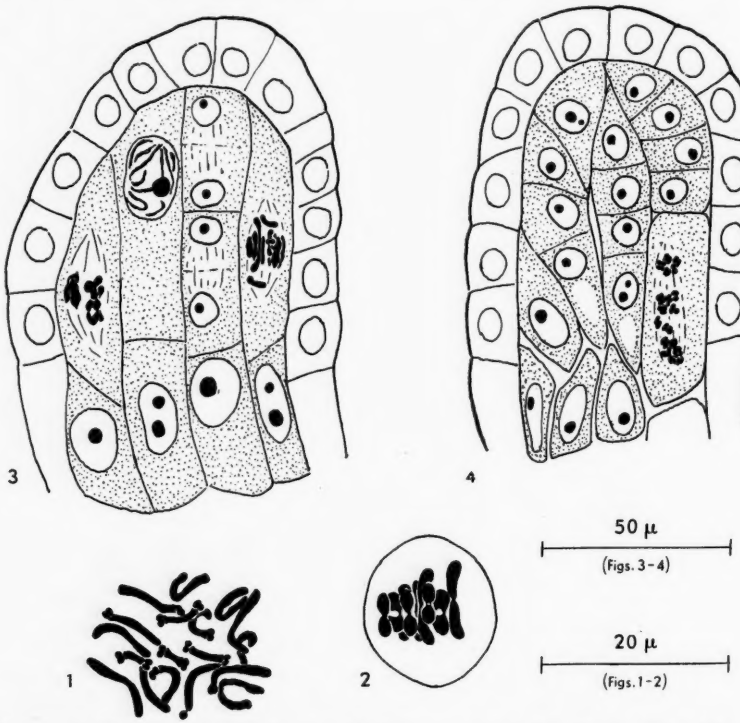
Concerning sporogenesis, I have collected material from three different plants and studied it carefully. Microsporogenesis appeared to be perfectly normal and a study of approximately hundred metaphases in each plant revealed nine bivalents in all PMCs (Fig. 2).

In the ovule the archesporium is multicellular, the number of archesporial cells varying between 5 and 8. Of the 79 metaphases-anaphases observed, 64 were normal. Fig. 3 shows an ovule with two such normal metaphases. The left one has been somewhat cut by the knife and exhibits only five bivalents. The other four lie, however, in the next section. The right metaphase contains all nine bivalents, one of which, however, has divided somewhat earlier than the others. The 15 remaining metaphases-anaphases were all abnormal. No bivalent formation occurred but all chromosomes were univalent. In these asyndetic divisions, the univalents were scattered at random on the spindle appearing in Fig. 4.

Thus, in the diploid plants total asyndesis occurred in about 19 % of the EMCs while all PMCs had an altogether normal meiosis. The

three investigated plants had about the same asyndesis frequency in their ovules.

Tetraploid Chrysanthemum carinatum. — In 1950 seeds from tetraploid *Chr. carinatum* were sown out and of the raised plants two were selected for a close investigation. Examination showed the chromosome



Figs. 1—4. Diploid *Chrysanthemum carinatum.* — 1, root tip mitosis ($2n = 18$). — 2, I-M in PMC with nine bivalents in side view. — 3, an ovule with EMCs in normal meiosis. — 4, an ovule with tetrads and one EMC in a total asyndetic meiosis.

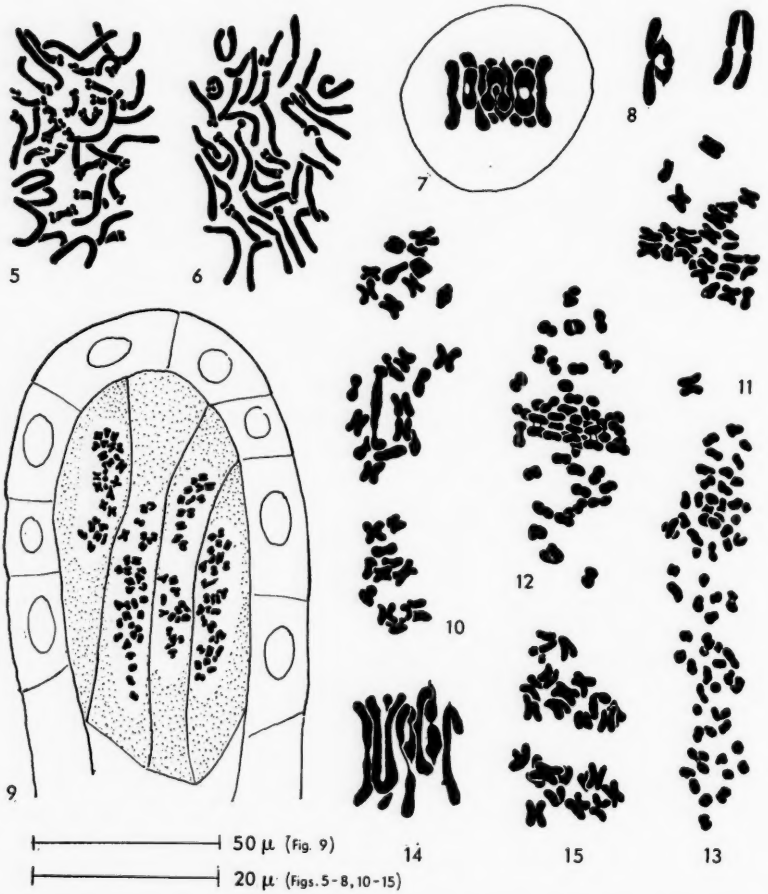
number of one to be $2n = 36$ (Fig. 5) and that of the other, $2n = 37$ (Fig. 6).

In both plants microsporogenesis is characterized by strong bivalent and polyvalent formation. Fig. 7 shows I-M in side view. Besides bivalents one trivalent is to be seen. In this PMC all chromosomes are not drawn. Fig. 8 shows two tetravalents from two different PMCs. Careful study of a great number of PMCs failed to show the occurrence

of asyndesis. Like the diploids, both plants have between 5 and 8 archesporial cells in their ovules. In macrosporogenesis asyndesis occurs as in the diploids, though with respect to frequency the two plants differ considerably from each other. In the 36-chromosome plant, a total of 95 metaphases-anaphases was observed, of which 72 were normal and 23 asyndetic. That is to say, the asyndesis frequency is 20,3 % or about the same as in the diploid.

In the 37-chromosome plant, a total of 109 metaphases-anaphases was observed, of which 24 were normal and 85 asyndetic. Consequently, the asyndesis frequency is no less than 70 %. This plant was especially favourable for a study of the asyndetic division; some cases are illustrated in Figs. 9—13. Fig. 9 shows an ovule with four EMC at I-A. All four are totally asyndetic, all 37 chromosomes, however, are not drawn, since the divisions have been somewhat cut by the knife. The remaining univalents lie, however, in the next section. In Fig. 10 a similar anaphase is illustrated from another ovule, but at a higher magnification. Apparently it deviates with regard to the fact that one or two bivalents occur. Bivalent formation in a limited extent sometimes occurs but, as a rule, these divisions are totally asyndetic. The longitudinal division of the univalents also seems to be very much advanced which makes them appear double. Generally the univalents are scattered at random on a very much elongated spindle; sometimes, however, a lot of them can be accumulated at the metaphase plane forming there a ± regular plate, as seen in Fig. 11 (all chromosomes are not drawn). Fig. 12 represents a similar case. Here, however, no less than fifty univalents can be distinguished, some of which (at the metaphase plane) give the impression of being completely divided. In this case the supernumerary chromosomes may have been formed by a metaphase-anaphase separation of some of the univalents into daughter univalents. However, turning to Fig. 13 another interpretation is equally possible. In Fig. 13 a very elongated anaphase appears with about seventy chromosomes. These are considerably smaller than the corresponding ones in, for example, Fig. 10 and no doubt are daughter univalents being formed at some prophase stage by division of univalents. I have encountered about ten such cases in different ovules of this plant. Consequently, it is very likely that the division illustrated in Fig. 12 may be a combination of the divisions in Figs. 11 and 13. The supernumerary chromosomes in Fig. 12 should then be daughter univalents being formed at prophase by division of some of the univalents.

It is to be expected that most asyndetic anaphases should give



Figs. 5—15. *Chrysanthemum carinatum*. Two tetraploid plants with $2n = 36$ (5) and $2n = 37$ (6—15). — 5—6, root tip mitoses with 36 and 37 chromosomes, respectively. — 7, I-M in PMC with bivalents and one trivalent. — 8, two tetravalents from two PMCs. — 9, an ovule with four EMCs at total asyndetic I-A. — 10, EMC at asyndetic I-A with 1—2 bivalents. — 11, asyndetic EMC with univalents forming an irregular metaphase plate. — 12, asyndetic EMC with about fifty univalent chromosomes some of which have formed a metaphase plate. — 13, EMC at asyndetic I-A with about seventy chromosomes, probably originating from univalents which have divided into daughter univalents at some prophase stage. — 14, EMC at I-M with bivalents and multivalents in side view. — 15, I-A in an EMC with normal meiosis. — All chromosomes are not drawn in Figs. 7, 9, 11—15.

rise to restitution nuclei. Not a single nucleus of that type has, however, been observed but the anaphase seems to be the final stage of this division. Degeneration sets in quite soon, the chromosomes clump together forming an elongated dark-coloured body. It is only EMCs with a normal meiosis which can give rise to viable daughter cells. The metaphase of such an EMC is illustrated in Fig. 14; as in the PMCs it is characterized by a strong bivalent and polyvalent formation. I have also drawn the anaphase (Fig. 15) following immediately after so that a comparison may be made with the corresponding stage in some of the asyndetic divisions (Figs. 9, 10 and 13). On such a comparison the elongated asyndetic anaphase appears very clearly.

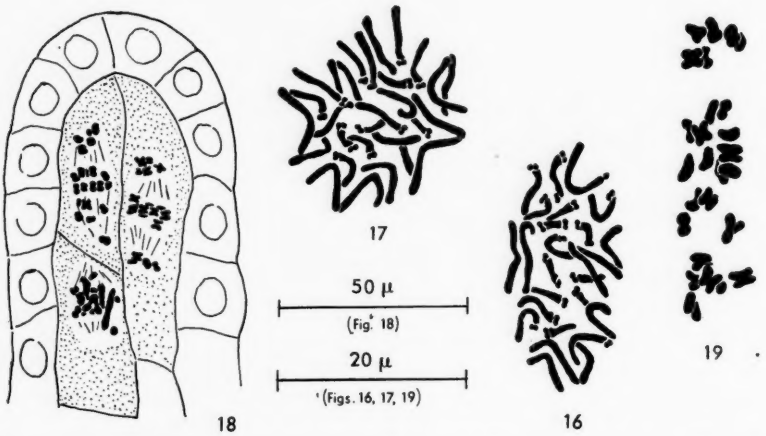
As a rule, the EMCs with normal meiosis give rise to four macrospores. Only rarely abnormalities in the form of micro nuclei, *etc.* are to be seen. One of the macrospores then forms the gametophyte which develops embryo and endosperm in a normal way. However, several ovules are naturally sterile, which especially applies to the 37-chromosomal plant.

Triploid Chrysanthemum carinatum. — In 1951 seeds from tetraploid *Chr. carinatum* were again sown out and by examining the chromosome number in root tips of about 80 plants two triploids were discovered. One had $2n = 26$ (Fig. 16) and the other $2n = 29$ (Fig. 17). All other plants had between 35 and 37 chromosomes. In 1950 the tetraploids and diploids were cultivated in close proximity to one another and therefore it is very probable that the two triploids have originated from the cross tetraploid $\times$ diploid.

In microsporogenesis the two triploids had a strong bivalent and trivalent formation. No asyndetic tendencies have been observed.

The triploid ovules, as the ovules of the diploids and tetraploids, have a multicellular archesporium with 5—8 archesporial cells. The two plants also coincide with the preceding ones with regard to meiosis. Thus, asyndesis of the same type as has been described above occurs, though with a somewhat different frequency. In the 26-chromosome plant 129 metaphases-anaphases have been observed, of which 54 were normal and 75 asyndetic. Thus, the asyndesis frequency in the EMCs is 41,9 %. In Fig. 18 an ovule of this plant is shown to have two asyndetic EMCs and a normal one with bivalent and trivalent formation. In Fig. 19 from another ovule I have drawn an asyndetic anaphase with 26 univalents. Asyndetic meiosis with tendencies to form metaphase plates has also been observed (Fig. 18) as well as five cases of the same type as appears from Fig. 13.

In the 29-chromosome plant 80 metaphases-anaphases have been observed and of these 64 were normal and 16 asyndetic. Consequently, the asyndesis frequency is 20 %, *i. e.* about the same as in the diploids and the 36-chromosome tetraploid. The asyndetic meiosis is identical with that of the preceding plant, except for the fact that anaphases of the type in Fig. 13 have not been met with.



Figs. 16—19. *Chrysanthemum carinatum*. Two triploid plants with $2n = 26$ and $2n = 29$. — 16—17, root tip mitoses with 26 and 29 chromosomes, respectively. — 18, an ovule with two asyndetic EMCs and a normal one. — 19, an asyndetic EMC at I-A.

Discussion. — Since BEADLE's (1930) investigation of asyndesis in *Zea mays* quite a lot of cases with asyndesis in different plants have been reported. A list of these is to be found in PRAKKEN (1943) with additions by JOHNSSON (1944) and ANDERSSON (1947). The list can be increased with two more cases, *viz.*, *Leontodon hispidus* (BERGMAN, 1935) and *Ulmus glabra* (EKLUNDH-EHRENBERG, 1949).

As to the causes of asyndesis, PRAKKEN has divided them into six groups. The present case, I suppose, belongs to the first one which is described by PRAKKEN (1943, p. 487) as follows: »Anaphasis due to the action of a distinct gene or genes (or some slight structural change)». By a quantitative increase of the chromosome number in *Chr. carinatum*, the asyndesis frequency alters only if the number of chromosomes also alters qualitatively. Thus, the diploid plants ($2n = 18$) have about the same asyndesis frequency as the tetraploid ($2n = 36$) while the 37-

chromosome and the 26-chromosome plants have quite another frequency. This indicates that asyndesis in *Chr. carinatum* is directly or indirectly genetically conditioned.

In the hitherto published investigations of asyndesis in various sexual plants often only PMCs have been studied. In those cases in which EMCs also have been examined, asyndesis on the ♂- and ♀-side has proved to be of about the same type. There are two exceptions to this rule, however. Thus, concerning *Viola Orphanides* CLAUSEN (1930, p. 71) reports: »Full conjugation between all chromosomes was observed in the female archespor of a pollen-sterile plant, together with practically complete absence of pairing between the chromosomes in the male archespor of the same plant». Furthermore, in *Rumex acetosa* ssp. *pratensis*, LÖVE (1943, pp. 358—359) reports a case with *y*-linked hereditary asyndesis. Hence, in *Viola* and *Rumex*, asyndesis only occurs in PMCs while EMCs have a completely normal meiosis. The contrary condition, *viz.*, asyndesis on the ♀-side and syndesis (bi- and multivalent formation) on the ♂-side is (as far as the present author knows) only established in the agamosperms. Consequently, among the sexual plants *Chr. carinatum* should be the first case with such a meiotical difference between micro- and macrosporogenesis.

SUMMARY.

(1) Didploid, triploid and tetraploid *Chrysanthemum carinatum* have been investigated with respect to meiosis in PMCs and EMCs.

(2) Three plants of the diploid ($2n=18$) were studied which proved to have normal meiosis in PMCs; in the ovules, however, total asyndesis occurred in about 19 % of the EMCs of each plant.

(3) Of the two plants of the tetraploid type examined, one proved to have $2n=36$, the other $2n=37$. Both had normal meiosis in PMCs with bivalents and multivalents. In the ovules the 36-chromosome plant had total asyndesis in 20,3 % of the EMCs (*i. e.* about the same as in the diploids). The 37-chromosome plant, on the contrary, had total asyndesis in no less than 70 % of the EMCs.

(4) Two plants of the triploid type were examined, one proving to have $2n=26$ and the other $2n=29$. Both had normal meiosis in PMCs with bivalents and trivalents. In the ovules the 26-chromosome plant had total asyndesis in 41,9 % of the EMCs and the 29-chromosome plant 20 % (*i. e.* about the same as in the diploids).

(5) The asyndetic meiosis stops at the anaphase stage and then degeneration begins. No restitution nuclei have been observed.

(6) All plants have 5—8 archesporial cells in the ovules. The ESs originate only from EMCs with normal meiosis of the same type as in corresponding PMCs. The ES-development is monosporic and the embryo is formed after fertilization.

(7) Asyndesis in *Chr. carinatum* is probably genetically conditioned and among the amphimicts this plant represents the first case with total asyndesis on the ♀-side and full syndesis on the ♂-side.

The author is very much indebted to Professor G. TURESSON of the Royal Agricultural College of Uppsala and to the members of his institute for material and valuable help.

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X-RAY INDUCED DOMINANT LETHALS IN DIFFERENT STAGES OF SPERMATOGENESIS IN DROSOPHILA

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INTRODUCTION.

IT is a well known fact that in *Drosophila melanogaster* the number of eggs that hatch is decreased when the sperm used in fertilizing the females has been previously X-ray irradiated. This decrease is not only due to the dose but also to the age of the males at treatment (DEMPSTER, 1941; STRÖMNÆS, 1949) and the time between treatment and fertilization (BONNIER and LÜNING, 1950). Possible explanations were given in the latter publication. The present paper deals with the forces at work altering the percentage of hatchability when increasing the time between treatment and insemination.

MATERIAL AND METHODS.

All females used were *y w sn* and in all series but two the males were wild type (Oregon). In the two series $sc^{S1}B$ *In-S* w^a sc^8 (MULLER-5) and *y w sn* males were used.

All cultures, experimental and stocks, were kept in incubator at 25° ± 1° C. Irradiations were made at the Radiophysical Institute by using a Siemens Stabilivolt apparatus of 155 kV, 6 mA with 1 mm Al. The dose was 2900 r given in 6 min. when there is no other statement. In one of the series different doses have been compared.

In the first part of the experiment the flies were mated in mass cultures with about 100 flies of each sex. In the later part they were mated in pair cultures. The mass cultures were kept in containers consisting of ordinary glass preserving jars with an exchangeable Petri dish with 1 % agar food, blackened by charcoal and, on the surface, a film of yeast-suspension. This food is called »black food». The pair cultures were kept in vials and supplied with a cube of black food on a cardboard parallelogram (SPENCER, 1943). The food was exchanged every day in both the mass and the pair cultures. So it was possible to determine the time from treatment of the males to egg laying.

The females were etherized once only, when collected. For use in mass cultures 200 females were placed in a vial for 3—5 days before mating. In order to get random differences among the females that were to be used the same day, all females were mixed and then without etherizing divided into groups of about 100. Females used in pair cultures were placed alone in vials for 3—5 days and then without etherizing transferred to the male.

In mass cultures the males were etherized when collected, when X-ray irradiated and when changing their mates. In pair cultures the males were etherized three times: when collected, when irradiated and when mated for the first time. They were not etherized when transferred to new females. The agar cube was not put into the vial until $1\,^1/_2$—4 hours after the males were brought together with their males. This reduced the possibility of getting unfertilized eggs.

When removed from the flies, the agar plates and cubes with the eggs were put into the incubator for 28 hours. After that the number of hatched and non-hatched eggs was counted under a dissecting microscope at a magnification of 20 ×.

No selection of the data was made except that cultures without any hatched eggs were excluded. This means that in cultures with a very low percentage of hatchability some cultures were excluded which probably were not quite sterile but randomly showed no hatched eggs. This is of no importance to the discussion.

RESULTS.

BONNIER and LÜNING (1950) showed that if newly hatched males are X-ray irradiated and mated 7 days later the percentage of hatchability was low irrespective of whether they are kept with or without females from treatment to mating for egg collection. For further studies of these phenomena a great number of 0—1 day old males were irradiated. After irradiation they were divided into four groups marked Ser. 1, 2, 3 and 4.

Ser. 1. — The males were subdivided into two portions and each mated to females in a preserving jar. At the beginning of the 7th day a. t. (= after treatment) they were transferred to new females.

Ser. 2. — The males were subdivided into two portions and each mated to females in a preserving jar. At the beginning of the 4th and the 7th day a. t. they were transferred to new females.

Ser. 3. — The males were kept in a vial without females until the

beginning of the 4th day a. t. They were then subdivided into two portions and each mated to females in a preserving jar. At the beginning of the 7th day a. t. they were transferred to new females.

Ser. 4. — The males were kept in a vial without females until the beginning of the 7th day a. t. They were then subdivided into two portions each mated to females in a preserving jar.

TABLE 1. *Series in which freshly hatched males were irradiated and then either mated at once or stored 3 or 6 days before mating.* ♀ = *the males were transferred to new virgin females.* E = *total eggs counted.* %H = *percentage of eggs hatched.*

Series	Days after treatment					
	2		5		8	
	E	%H	E	%H	E	%H
1	♀ 128	46,1	284	39,1	♀ 345	14,5
2	♀ 237	38,4	♀ 352	41,5	♀ 318	5,7
3			♀ 218	46,3	♀ 345	8,4
4					♀ 413	9,7

Eggs laid on the 2nd, 5th and 8th day a. t. were counted and the data are presented in Table 1. It is, as seen, of no importance if the males which were mated to virgin females on the 7th day a. t. were previously mated or unmated. The data further show that the percentage of hatchability is practically unaltered between the 2nd and the 5th day a. t. but on the 8th day there is a sharp decrease.

In order to investigate if this decrease depends on the dose a great number of 0—1 day old males were collected. Three groups of 100 males were used in control series. The other males were divided into groups and X-ray irradiated with the dose »1, 2, 3, 4, 5 and 6 minutes», where 6 min. was 2900 r-units. At the same time two other groups of males, 6—7 days old, were irradiated. The males in one of these groups were kept without and in the other with females from hatching to treatment. These groups were given the dose 6 min. In Table 2 these two series are marked 6′ (males kept without females) and 6″ (males kept with females). Immediately after treatment all males were mated in mass cultures. At the beginning of the 4th and the 7th day a. t. all males were transferred to new virgin females. After the 9th day only controls, 3, 6 and 6′ series were continued. In those series which were

TABLE 2. *Series in which males were irradiated and given different dosages. They were transferred to new females every third day. The eggs counted were from the second day of the females' laying.*

	Days after treatment													
Series	2		5		8		11		14		17		20	
(Dosage)	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H
Control	1151	92,5	1013	92,0	1102	92,5	1018	89,9	942	89,6	983	89,7	664	94,4
1	862	87,1	486	89,9	602	69,3								
2	584	82,4	526	78,9	579	44,0								
3	560	75,4	264	72,0	814	39,2	376	51,1	822	70,1	954	70,7	639	83,3
4	422	60,0	666	60,1	721	20,7								
5	752	58,0	505	44,4	646	16,4								
6	705	45,4	485	38,6	753	10,1	328	63,4	642	53,8	361	74,7	287	85,7
6′	676	39,3	376	39,4	719	32,8	626	40,6	466	68,0	858	79,8	682	90,2
6″	684	38,6	483	35,4	412	31,6								

continued the males were transferred to new females on the 10th, 13th, 16th and 19th day a. t. The percentage of hatchability was counted on plates from the 2nd day of each mating, accordingly on the 2nd, 5th, 8th, 11th, 14th, 17th and 20th day a. t. The data are presented in Table 2. In all series with treated males there is a more or less sharp decrease in the percentage of hatchability on the 8th day compared with the 2nd and the 5th day a. t. If these frequencies are plotted against the dose on a logarithmic scale they are linear on the 2nd as well as on the 8th day but with different slopes. This indicates that in both cases single breaks were the cause of the dominant lethals, and that the frequency of these was much greater in the sperm that fertilized the eggs on the 8th day a. t. than on the 2nd day. On the 11th day a. t. there was an increase in the percentage of hatchability. On the 20th day the effect of the X-ray treatment was negligible.

Up to now it has been shown that between the 5th and the 8th day a. t. of 0—1 day old males there was a lowering in the percentage of hatchability. In this process the age of the males has increased automatically with the time from treatment. For this reason it is not possible to decide if it is the age of the males or the time from treatment that is the cause of the lowering of the hatchability. In order to solve this problem males were collected every day for 6 days and kept without females until irradiation, which was made on the very day the last males were collected. The age of the last collected males was 0—1 day at treatment. They are presented in Table 3 as group 1. Those collected the day before treatment were 1—2 days old at treatment. They are called group 2. 2—3 days old males are called group 3 and so on to

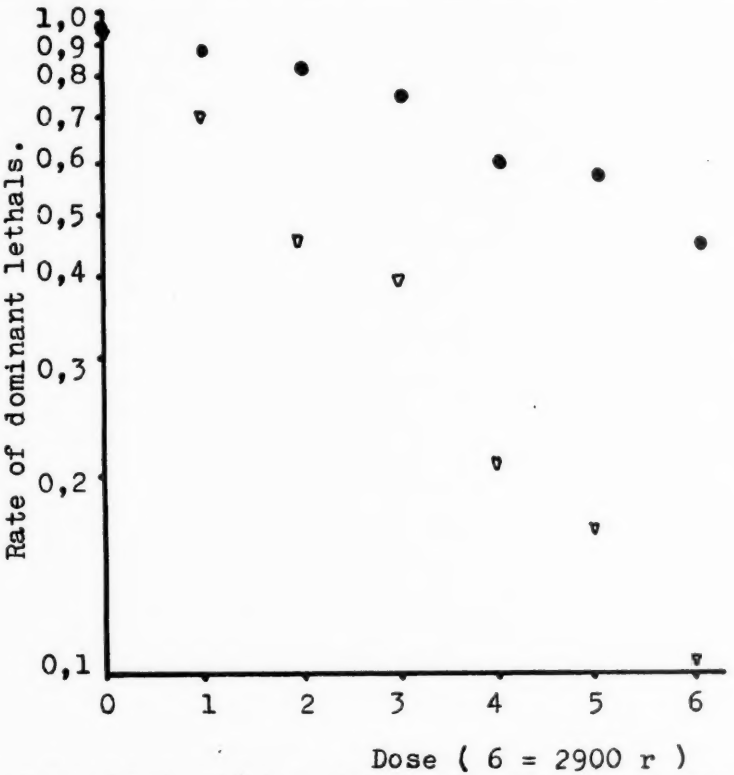

Fig. 1. Rate of dominant lethals as a function of dose. The rate on a logarithmic scale. ● = eggs fertilized during the 2nd day and ▽ = eggs fertilized during the 8th day after treatment of the males.

group 7, which consists of 6—7 days old males. After treatment all groups were divided into portions. From group 1 it was 7 portions; from group 2 it was 6 portions, and so on to group 6, that was divided into 2 portions. Group 7 was not divided. From each group one portion was immediately mated in mass cultures subdivided into two containers. Those portions that were not mated on the same day as treatment, were kept without females in vials. Every day a new portion of each remaining group was mated in the same manner until the 6th day a. t. when there remained only one portion from group 1. In each portion the egg count was made for two consecutive days. A total of 39426 eggs were counted. The results are presented in Table 3 where *a* represent data

TABLE 3. *Males of different ages (group 1 = 0—1 day; group 2 = 1—2 days; ... group 7 = 6—7 days old) were irradiated and then mated at*

Group	Days after treatment						
	1	2	3	4	5	6	7
1	31,9	47,9	51,1	44,4	30,1	16,8	10,1
2	42,4	44,2	46,5	48,0	35,0	23,6	
3	37,5	39,5	42,8	42,0	32,1		
4	33,0	43,0	49,0	44,3			
5	35,6	34,8	49,0				
6	29,7	36,4					
7	32,9						

a

from eggs collected during the first day and *b* data collected during the second day after mating. From the table we infer that the low percentage of hatchability between the 5th and the 8th day a. t. is not due to the absolute age of the males, as suggested by BONNIER and LÜNING (1950), but appears more or less sharp in a certain time after treatment with its greatest effect in those males which were youngest at treatment. Unfortunately it was not possible to continue all groups up to the 7th day a. t. Thus it is not possible to give an absolute statement about the effect on the males that where up to 7 days old at treatment. For that reason some experiments were later carried out with 6—7 days old males. See Table 4, Ser. 10, 11 and 12. The results of these series will be discussed below.

To determine more closely when the lowering of the percentage of hatchability appears, and if it appears by leaps or continuously, some new experiments were carried out. The data from these are collected in Table 4. To facilitate the discussion some figures from Tables 2 and 3 are included. For considerations of space it was not possible to give an analysis of single males. To show the degree of variation the data for

different times after irradiation. Eggs collected first day after mating in table a and second day in table b.

Group	Days after treatment						
	2	3	4	5	6	7	8
1	44,8	49,8	49,7	40,7	28,1	13,6	18,0
2	45,5	50,2	53,5	49,7	32,8	24,4	
3	36,7	40,4	46,3	44,5	31,5		
4	37,1	50,6	43,7	49,5			
5	35,6	45,5	39,1				
6	39,6	45,4					
7	36,5						

b

single males are, however, included in Tables 5 and 6. Table 5 corresponds to Ser. 7 of Table 4, and Table 6 to Ser. 10 of Table 4.

Table 4 contains:

Ser. 1. — The same as Ser. 6 of Table 2. (3561 eggs.)

Ser. 2. — A repetition of the preceding series. (8428 eggs.)

Ser. 3. — 0—1 day old males were X-ray irradiated. 20 of them were mated singly in vials, each with a single female. These females were kept together with their mates during the whole experiment. (7598 eggs.)

Ser. 4. — 40 males of the same group as Ser. 3 were mated singly in vials each with a single female. The males were transferred to new females every day. (8981 eggs.)

Ser. 5. — After separation of the males from the females in Ser. 4 these females were kept in separate vials with agar food containing honey instead of molasses to impede egg laying. Those females which were inseminated on the 2nd, 3rd, 4th, 5th and 6th day a. t. of the males, were given black food at the beginning of the 7th day a. t. and were allowed to lay eggs for one day. (These females gave in all 1625 eggs.)

TABLE 4. *For information of the different series, see the*

Series	Age of males at treatment	Days after treatment									
		1	2	3	4	5	6	7	8	9	10
1	0—1 day	♀	45,4		♀	38,6		♀	10,1		♀
2	0—1 »	♀	41,7		♀	34,0		♀	5,2		♀
3	0—1 »	♀ 43,1	43,7	42,7	53,2	54,6	38,0	32,6	29,0	27,8	
4	0—1 »	♀ 35,1	♀ 48,2	♀ 52,4	♀ 46,4	♀ 33,8	♀ 14,4	♀ 5,2	♀ 4,1	♀ 6,9	
5	Females from ser. 4 on honey agar food							12,9			
								35,5			
								35,1			
								55,5			
								41,7			
6	0—1 day (kept without females to egg collection)	♀ 31,9									
			♀ 47,9								
				♀ 51,1							
					♀ 44,4						
						♀ 30,1					
							♀ 16,8				
								♀ 10,1			
7	$< 2^{40}$ hours	♀ —	♀ 54,3	♀ 55,3	♀ 43,6	♀ 38,7	♀ 4,2	♀ 3,6	♀ 3,7	♀ 6,6	♀ 12,0
8	Pupae hatching:										
	0—1 d. a. tr.		♀ 50,2	♀ 47,0	♀ 40,2	♀ 18,3					
	1—2 »			♀ 50,8	♀ 22,1	♀ 8,6					
	2—3 days after treatment				♀ 9,0	♀(8,3)					
9	7—8 days	♀	39,3		♀	39,4		♀	32,8		♀
10	6—7 »	♀ 36,5	♀ 33,8	♀ 40,9	♀ 41,3	♀ 34,8	♀ 12,6	♀ 12,8	♀ 6,8	♀ 14,8	♀ 17,2
11	6—7 »						♀ —	♀ 25,1			
12	6—7 »								♀ 22,2	♀ 20,4	
13	0—1 day							♀ 5,4	♀ 3,9		
14	0—1 »									♀ 4,1	♀ 7,7
15	0—1 »										
16	0—1 »										
17	0—1 »										

Ser. 6. — The same as group 1 of Table 3 *a*. (3630 eggs.)

Ser. 7. — 20 males, which at the beginning of the treatment were not more than 2 hours 40 minutes old, were mated singly in vials each with a single female. The males were transferred to new females every day. (6207 eggs.)

Ser. 8. — Pieces of tissue paper with pupae of different ages were

text. (♀ = *the males were transferred to new females.*)

Days after treatment												
11	12	13	14	15	16	17	18	19	20	21	22	23
63,4		♀	53,8		♀	74,7		♀	85,7			
28,7		♀	74,1		♀	84,2		♀	89,0		♀	84,4
♀ 66,6	♀ 74,9											
40,6		♀	68,0		♀	79,8		♀	90,2			
♀ 56,6	♀ 63,8											
♀ 15,1	♀ 22,9											
		♀ 33,6	♀ 36,1									
				♀ 50,6	♀ 62,1							

taken from some bottles of wild type stock. The pieces were irradiated and then placed in fresh bottles. Newly hatched males were collected every day a. t. They were immediately mated singly in vials, each with a single female and transferred every day to new females. Females inseminated by males that hatched 0—24 hours a. t. gave 1626 eggs. Those inseminated by males that hatched 24—48 hours a. t. gave 1102

TABLE 5. *Data of individual males from Ser. 7, Table 4. Males irradiated at an age of* $< 2^{10}$ *hours and immediately mated in pairs and transferred to new females every day.*

Male	Days after treatment																					
	2		3		4		5		6		7		8		9		10		11		12	
No.	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H
1	57	64,9	53	54,8	50	36,0	84	27,4	76	5,3	81	1,2	42	2,4	52	1,9			47	83,0	64	90,7
3	48	47,9	47	61,7			52	50.0	42	4,8			54	3,7	92	1,1			45	75,7	83	94,0
5															87	3,4					44	25,0
6	48	41,7	46	52,2			63	34,9	71	1,4			38	5,3	79	19,0			77	89,7	88	87,5
7	56	37,5	40	47,5	33	36,3	68	23,5														
8	71	55,3	37	54,0																		
9	87	47,1	60	46,7	39	41,1	63	22,2	76	6,6	77	2,6					64	3,1	44	4,5	83	12,0
10	48	55,3	44	54,6	42	54,8	74	27,0														
11	72	59,8	41	63,4			34	41,2							78	9,0	87	13,8	43	60,4	73	93,2
12	86	57,0	42	50,0	61	49,2	52	19,2							56	1,8					64	73,5
14	82	53,7	36	63,9			76	31,6							108	2,8	82	3,7	59	25,4	77	23,4
15			43	55,8			49	14,3							82	3,7			61	85,2	85	85,9
17	29	68,9	52	61,6	18	38,9	56	17,9			78	5,1					63	7,9	70	55,7	68	92,7
18	47	51,1	32	37,5			42	47,7			70	5,7			105	9,5	55	36,4	48	93,8	73	95,8
19			50	66,0			65	27,7							58	8,6	70	14,3	59	76,3	78	92,3
20	70	65,7	61	55,7			73	27,4	65	3,1							69	10,1	64	70,3	74	94,6
Total	801	54,3	684	55,3	243	43,6	851	28,7	330	4,2	306	3,6	134	3,7	797	6,6	490	12,0	617	66,6	954	74,9

TABLE 6. *Data of individual males from Ser. 10, Table 4. Males irradiated at an age of 6—7 days and immediately mated in pairs and transferred to new females every day.*

Male No.	1		2		3		4		5		6		7		8		9		10		11		12	
	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H
1					37	32,4	42	35,7	55	29,1	80	8,8			60	5,0	85	5,9	78	23,1	62	37,1	57	38,6
2	50	42,0	74	29,7	40	45,0																		
3	50	42,0			20	35,0			50	34,0					42	14,3	64	6,3	79	16,5	51	53,0	61	31,2
4	53	34,0	73	34,2	36	50,0																		
5	64	32,8	37	27,0																				
6	55	34,6			58	39,7	18	38,9	45	60,0			52	13,5	47	6,4	91	9,9	81	8,6	56	33,9	90	26,7
7	39	48,7	68	22,1	47	34,0	50	42,0	59	45,8			47	14,9	49	2,0	76	26,3	81	27,2			64	86,0
8	67	25,4	58	48,3	42	31,0			49	36,8							105	11,4	39	12,8	54	63,0	55	81,8
9	80	33,8	82	33,0	37	51,4	26	50,0	74	32,5	46	6,5	52	5,8					75	2,7	62	40,3	63	57,2
11			54	24,1	34	29,4			35	40,0	36	36,1					67	13,4	67	9,0				
12	59	37,3	71	43,7	66	47,0			60	41,7							49	32,6	62	14,5	60	35,0	61	55,7
13	47	34,0	84	39,5	49	44,9	30	36,6	47	21,3			67	16,4					86	31,4	54	77,8	68	92,7
14	48	41,7			19	42,1	46	43,5	61	42,6					51	2,0					61	90,2	85	87,1
15	59	47,5			40	37,5			51	35,3					45	13,3								
16	60	35,0			36	38,9			52	38,5														
17	40	35,0																						
18					43	46,5																		
19					46	50,0			57	47,4														
20	56	32,1			47	36,7	47	42,6			53	7,6	55	12,7			58	22,4	19	31,6	49	85,8	50	90,0
Total	827	36,5	601	33,8	692	40,9	259	41,3	695	34,8	215	12,6	273	12,8	294	6,8	595	14,8	667	17,2	509	56,6	654	63,8

eggs and those inseminated by males that hatched 48—72 hours a. t. gave only 191 eggs. With increasing time between treatment and hatching there was an increased sterility.

Ser. 9. — The same as Ser. 6′ of Table 2. (4403 eggs.)

Ser. 10. — 6—7 days old males were irradiated. 20 of them were mated singly in vials, each with a single female. The males were transferred every day to new females. (6281 eggs.)

Ser. 11. — Males from the same group as Ser. 10 were kept without females until the 6th day a. t. when 20 of them were mated singly in vials, each with a single female. After one day the males were transferred to new females. On the 6th day egg collecting failed. (367 eggs.)

Ser. 12. — Males from the same group as Ser. 10 and 11 were kept without females until the 8th day a. t. when 20 of them were mated singly in vials, each with a single female. After one day the males were transferred to new females. (1272 eggs.)

Ser. 13. — 0—1 day old males were irradiated and then kept without females until the 7th day a. t. when 20 of them were mated singly in vials, each with a single female. After one day the males were transferred to new females. (1062 eggs.)

Ser. 14. — Males from the same group as Ser. 13 were kept without females until the 9th day a. t. when 20 of them were mated singly in vials, each with a single female. After one day the males were transferred to new females. (1473 eggs.)

Ser. 15. — Males from the same group as Ser. 13 and 14 were kept without females until the 11th day a. t. when 20 of them were mated singly in vials, each with a single female. After one day the males were transferred to new females. (1422 eggs.)

Ser. 16. — Males from the same group as Ser. 13, 14 and 15 were kept without females until the 13th day a. t. when 20 of them were mated singly in vials, each with a single female. After one day the males were transferred to new females. (1157 eggs.)

Ser. 17. — Males from the same group as Ser. 13, 14, 15 and 16 were kept without females until the 15th day a. t. when 20 of them were mated singly in vials, each with a single female. After one day the males were transferred to new females. (1144 eggs.)

In all the experiments hitherto presented there was a decrease in the hatchability about the 6th day a. t. To test if this was a peculiarity in the stock used or if it was a common phenomenon two other stocks were used, viz. *sc*^{S1}*B In-S w*^{a} *sc*^{s} (MULLER-5) and *y w sn*. Males of both stocks were irradiated at an age of 0—1 day and immediately mated

TABLE 7. *0—1 day old* $sc^{S1}B$ *In-S* w^a sc^8 (MULLER-5) (*Table 7 a*) *and y w sn* (*Table 7 b*) *males were irradiated and immediately mated in pairs. At the beginning of the 7th day after treatment the males were transferred to new females.*

Days after treatment				Days after treatment			
2		7		2		7	
E	% H	E	% H	E	% H	E	% H
294	34,4	265	9,1	494	47,2	364	6,9
a				*b*			

TABLE 8. *Control series. 0—1 day old males mated in pairs and transferred to new females every day.*

Age of males in days															
1		2		3		4		5		6		7		8	
E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H	E	% H
382	94,0	832	90,4	901	91,9	1068	93,0	738	87,0	611	86,4	457	86,0	913	86,3

singly in vials, each with a single female. At the beginning of the 7th day a. t. the males were transferred to new females. Eggs were counted on the 2nd and the 7th day a. t. The results are presented in Table 7 *a* and *b*. These results and those from the wild type males agree completely.

Table 8 summarizes the results from a control series, where untreated 0—1 day old wild type males were mated singly in vials, each with a single female. The males were transferred to new females every day. There was apparently no considerable variation during the 8 days.

DISCUSSION.

BONNIER and LÜNING (1950) showed that when lengthening the time from irradiation to fertilization the rate of dominant lethals increases. In the present paper this phenomenon is further studied. Summing up the results from all series, one may state that between the 5th and 7th day after treatment there is a more or less sharp increase in the rate of dominant lethals. Up to the 11th day a. t. the high rate of dominant lethals remains nearly constant. On the 11th day there is, at intensive mating, a very sharp decrease in the rate of dominant lethals until the 20th day a. t. when the rate of dominant lethals is nearly the same as in the control.

DEMEREC and KAUFMANN (1941) studied the time required to exhaust the sperm which was mature at irradiation. They gave the follow-

ing definition of mature and immature sperm: »By immature we mean the sperm which was at such a stage of development at the time of treatment that it gives a smaller percentage of dominant lethals when used subsequently in copulation than does sperm which we call mature». According to this definition and to the present data, stages immature at treatment would be used earliest on the 11th day a. t.

In order to have a cytological background for the discussion and, if possible, also to give the words »mature» and »immature» a cytological meaning, some data will be given about the spermatogenesis according to COOPER's summing up in »Biology of *Drosophila*» (1950): At the time of pupation the spermatocytes start meiosis. Sperm is ready about 24—30 hours after pupation. The absolute number of cells in meiosis reaches its maximum in the middle of the pupal stage. At hatching the testes contain mainly spermatozoa and spermatids but also spermatocytes and spermatogonia. The metamorphosis of spermatids to spermatozoa involves at first an enlargement of the nucleus and then, in connection with lengthening, a concentration of the chromatin to one side of the nucleus and gradually a decrease of the volume.

By using the strict sense of the word maturation DEMEREC and KAUFMANN's (1941) definition of immature stages includes spermatocytes and spermatogonia. But it seems to be incorrect to include both spermatids and spermatozoa among mature stages. So I propose to change their definition reckoning spermatids to a *maturing* stage and spermatozoa to a *mature* stage.

If stages immature at treatment can be used earliest on the 11th day after treatment, stages maturing and mature at treatment are consequently supposed to be exhausted within 10 days. To explain the differences in the rate of dominant lethals in this period the following hypothesis will be put forth: In the first 5—7 days after irradiation the males ejaculate sperm which is mature at treatment and in the next 3 or 5 days they ejaculate sperm which is maturing at treatment and more »sensitive».

To test this hypothesis pupae were irradiated. One would expect that the earlier the pupae are treated the smaller would be the number of treated mature spermatozoa, and the fewer these are, the more rapid would they be exhausted. There is a good degree of agreement between this expectation and the results obtained. (Ser. 8, Table 4.)

It has been supposed by DEMEREC and KAUFMANN (1941) that spermatozoa of unmated males may be retained for many days. The present data indicate, however, that this is not the case. Comparing

Ser. 4 and 7 *versus* 6, 13—17 in Table 4 it is seen that it is insignificant if the males are repeatedly mated or kept without females from treatment up to 14 days after treatment. The increase of dominant lethals appears on the same day in both cases. From this we may conclude that if the males are not allowed to mate the ready sperm is resorbed and/or ejaculated without copulation. This does not mean that there may not exist minor differences in the number of mature spermatozoa in unmated males of different ages. At irradiation of 6—7 days old males the increase in dominant lethals is not so sharp if the males have not been mated intensively.

A consequence of the resorption and/or ejaculation without copulation is that within the testes there is no mixing of spermatozoa which matured at different times. If there was a mixing one should get results resembling Ser. 3, Table 4 where the same females and male were kept together for 9 days. In the females sperm inseminated at different occasions will mix. When the males are kept without females for some days after treatment and then mated they do not give the same results as Ser. 3.

Hitherto I have tried to show that the periods of different rate of dominant lethals should fit in with the different stages in spermatogenesis. But how to explain the high rate of dominant lethals when treating spermatids?

To explain the increasing rate of dominant lethals BONNIER and LÜNING (1950) tentatively suggested that in spermatozoa the average distance between the two ends of the broken chromosome increased with the time from treatment to fertilization, and so the possibility of restitution is reduced. If this suggestion is modified to mean breaks occurring in spermatids it should possibly explain the very high frequency of dominant lethals. If breaks occur in the chromosomes of the spermatids one would *a priori* expect them to separate when the chromosomes move, following changes in the volume of the nucleus. Using LEA and CATCHESIDE's (1945) calculations of the mean frequency of breaks and supposing the same frequency of breaks in spermatozoa and spermatids, about 11,5 per cent of the fertilizing spermatozoa would have no breaks. This number is greater than the frequency of hatched eggs from the 6th to the 10th day a. t. Consequently all breaks in the spermatids must lead to dominant lethals. Thus one would not expect any changes in the chromosome set in flies fertilized by sperm inseminated in that period. In some experiments, now being carried out, the rate of point mutations (*y*, *w* and *sn*), gynandromorphs and hyper-

ploid males in the two periods 1—6 and 7—10 days after treatment, have been studied. There is significantly more point mutations and hyperploid males among flies hatching from eggs fertilized, by spermatozoa inseminated 7—10 days after treatment than when they are inseminated 1—6 days after treatment. This is contrary to expectation if the rate of breaks is the same in spermatozoa and spermatids. So one must expect a higher probability of breaks in spermatids than in spermatozoa. This does not, however, exclude a higher probability of broken ends of chromosomes moving apart and not restituting during the transformation of spermatids to spermatozoa.

A few words may be said about a tentative hypothesis which has been rejected. The increase in dominant lethals could be due to protracted changes in sperm. That this is not the case is shown by Ser. 5, Table 4, where females inseminated at different times after the treatment of males were allowed to lay eggs at the 7th day after treatment. There was no increase when ageing the sperm in the females. AUERBACH (1950) obtained the same result when treating males with mustard gas.

Further studies about recessive lethals and their connection with chromosomal changes and translocations are planned.

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SUMMARY.

(1) Wild type, $sc^{S1}B$ *In-S* w^a sc^8 (MULLER-5) and *y w sn* males of *Drosophila melanogaster* were irradiated with 2900 r and mated immediately or after some days in mass or in pair cultures to *y w sn* females. In most series the males were transferred to new females every single or every third day.

(2) In the first 5 days after treatment the rate of dominant lethals is practically constant. There is then a more or less sharp increase in the rate, which remains until the 11th day when there is a sharp decrease which continues to the 20th day.

(3) Males irradiated at an age of 0—1 day showed a sharper increase in the rate of dominant lethals than males which were 6—7 days at treatment.

(4) To explain the variation in the rate of dominant lethals it is supposed that it depends upon the stage during spermatogenesis at which the presumptive spermatozoa are treated. Accordingly, spermatozoa used during the first 5 days after treatment were *mature* at treatment; during the next 5 days the spermatozoa used were *maturing* (spermatids) at treatment, and from the 11th day after treatment the spermatozoa used were in spermatocyte or spermatogonial stages at treatment.

(5) It is shown that the frequency of breaks is greater in spermatids than in spermatozoa.

(6) It is shown that, when the males are unmated, the spermatozoa are resorbed and/or ejaculated without copulation.

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ABSTRACTS

K. G. LÜNING: X-ray induced mutations in *Drosophila melanogaster.*

When irradiating males of *Drosophila melanogaster* it was shown (LÜNING, 1952) that about 6 days after the treatment there was a more or less sharp increase in the rate of dominant lethals. It was supposed that spermatozoa mature at treatment were used in the first 6 days and that from the 6th to the 11th day spermatozoa in a spermatid — maturing — stage at treatment were used. Supposing the same frequency of breaks in spermatozoa and spermatids, it was shown that the rate of dominant lethals in spermatids was so high that all breaks must lead to dominant lethals and, consequently, there would not occur any mutations in the viable sperm. To test this the following series were carried out. Wild type males were irradiated at different ages and mated to *y w sn* females at different times after treatment. The normal offspring were *y w sn* males and heterozygous *y w sn*/+ females. Among the normal offspring there appeared a low frequency of aberrant flies. The females were carefully examined and point mutations (*y*, *w* and *sn*) and gynandromorphs were registered. Among the males there appeared *w sn* and *sn* males and in one case a *w* male. These were classified as hyperploid males. They had derived a normal X-chromosome from their mother and a small part of the paternal X-chromosome. The results are summarized in the table below.

Ser. 64. — 0—1 day old wild type males were X-ray irradiated with 960 r and immediately mated to *y w sn* females in vials.

Ser. 65. — The same males as in Ser. 64 where transferred to new *y w sn* females at the beginning of the 7th day after treatment.

Ser. 74. — The same males as in Ser. 64 and 65 were transferred to new *y w sn* females at the beginning of the 11th day and remained there until the 14th day after treatment, when they were discarded.

Ser. 75. — 6—7 days old wild type males were X-ray irradiated with 960 r and immediately mated to *y w sn* females.

Ser. 84. — The same males as in Ser. 75 were transferred to new *y w sn* females at the beginning of the 7th day after treatment. At the 11th day they were discarded.

Series	Total females and gynandromorphs	Mutations	Gynandromorphs	Hyperploid males
64	59856	8	35	12
65	62479	26	41	65
74	14165	0	1	1
75	48336	18	22	14
84	39035	15	13	30

The females were allowed to lay eggs only during the period they were with there mates. The offspring were reared on tissue paper moistened in yeast suspension. All cultures were kept in incubator at 25° ± 1° C.

From the table we infer that the rate of hyperploid males was much lower

in the first 6 days than in the following 4 days. The rate of dominant lethals showed a similar variation. Is there any connection between dominant lethals and hyperploid males? The former are supposed to be due to unjoined breaks and asymmetrical interchanges. Hyperploid males are deficient in the greater part of an extra X-chromosome and, consequently, they may be classified as asymmetrical interchanges that happened to be viable because the extra X-chromosome segment was too short to disturb the development severely. That the viability of hyperploid males is due to the length of the extra X-chromosome segment is illustrated by the fact that there are 113 *w sn* : 8 *sn* males in the present material. The high rate of hyperploid males from treated spermatids indicates that the high rate of dominant lethals in the same period is partly due to more asymmetrical interchanges. From this we may conclude that in spermatids one must suppose a higher rate of breaks than in mature spermatozoa. This will be further studied. It seems as if 960 r will induce as many breaks in spermatids as 3000—4000 r in mature spermatozoa.

Strange to say, there is no increase in the rate of gynandromorphs when the rate of hyperploid males increases. BONNIER and LÜNING (1951) showed that when the dose was increased the gynandromorphs increased linearly and that hyperploid males increased as $(\text{dose})^{3/2}$. If there are more breaks in spermatids one would suppose that either the rate of gynandromorphs would increase in proportion to the number of breaks or decrease if the high rate of dominant lethals was due to movements of the chromosomes separating the broken ends. But one would hardly expect that the rate would remain constant. To explain this the following hypothesis is proposed: Breaks leading to gynandromorphism are induced as potential breaks which are not separable by movements of the chromosomes, but which break up in the next chromosome division. Thus, they are independent of breaks leading to dominant lethals.

Finally some words may be said about point mutations (*y*, *w* and *sn*). When irradiating newly hatched males the rate was significantly lower than when irradiating 7 days old males or irradiating spermatids in newly hatched males. The same result but not so striking was obtained by BONNIER and LÜNING (unpublished). Up to now I do not know how to explain this peculiarity.

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GERT BONNIER, K. G. LÜNING and BARBRO ARNBERG: On a possible mutagenic effect of x-rayed egg cytoplasm.

Several experiments made by different investigators during the last few years indicate that the mutagenic effect of irradiation is not a direct one, or at least not wholly so (BAKER and SGOURAKIS, 1950; THODAY and READ, 1949; GILES and RILEY, 1950; WYSS, HAAS, CLARK and STONE, 1950). From this it

could be supposed that x-raying the cytoplasm of unfertilized eggs would induce mutations in the paternal chromosomes which are introduced at a subsequent fertilization. WHITING (1950) did, however, not find such a result in *Habrobracon*. In spite of this, experiments were undertaken during the spring of 1951 with *Drosophila melanogaster*.

In one series (series Bd), 7 days old virgin *y w sn* females were x-rayed with a dose of 2400 r and immediately thereafter mated to *M5* males, 1—5 days old. Concurrently, a control series (series Be) was run, the only difference being that the *y w sn* females were untreated (*M5* = Muller 5 = $sc^{S1}B$ *In-S* w^a sc^8). In both series heterozygous *y w sn*/*M5* females were mated individually for testing the occurrence of sex linked lethals. When, in the progeny of such a female, the sons were of only one kind, *y w sn* or *M5*, the case was recorded as a lethal mutation, but only so if there were at least 15 males of the surviving type. If there were less, a new generation was in many instances secured and the case was recorded as a mutation if in the two generations, taken together, at least 15 males of the surviving type were found. Cultures giving only one male type but not fulfilling these requirements were omitted. (The same principle is followed in all experiments reported in this paper.) The results are shown below.

Series	Dose	Total chromosomes tested	Lethals in maternal chromosomes	Lethals in paternal chromosomes
Bd	2400 r	6537	178 = 2,72 %	13 = 0,20 %
Be	control	6861	10 = 0,15 »	12 = 0,17 »

It is obvious that the rate of paternal lethals equals the spontaneous rate.[1] A new series (series Bf) was started in which 0—2 days old *y w sn* females were mated to 0—1 day old *M5* males. Two days later the males were taken from the females and, still one day later, these females were, without etherization, given 960 r. Eggs were collected 3 hours and 8 hours after irradiation.

Series	Hours from irradiation to egg laying	Total chromosomes tested	Lethals in maternal chromosomes	Lethals in paternal chromosomes
Bf	0—3	81	1 = 1,23 %	3 = 3,70 %
	3—8	753	11 = 1,46 »	26 = 3,45 »
Total	0—8	834	12 = 1,44 %	29 = 3,48 %

No difference is found between 0—3 and 3—8 hours so, for later comparisons, we will use the total of 0—8 hours. There is, in series Bf, a much higher incidence of mutations in the paternal than in the maternal chromosomes. This is supposedly, at least partially, due to eliminations of aberrant chromosomes during female meiosis. It seems, however, that a percentage of 3,48 of paternal mutations is rather high for a dose of 960 r. (At 1000 r, SPENCER and STERN,

[1] A similar result was reported by Dr. W. BAKER at the Cold Spring Harbor Symposium in June, 1951.

1948, found a rate of 2,11 % with an upper limit of 2,47 %.) To check this, a new series (series Bq) was started. 3—4 days old *M5* males were treated with 960 r. Within 10 minutes after irradiation the males were transferred to 3—5 days old virgin *y w sn* females and eggs were collected 8 hours later. The whole difference between series Bf and Bq is thus that the spermatozoa were irradiated within the females in series Bf but within the males in series Bq. After the 8 hours elapse the females were, in order to minimize egg laying, transferred without males to honey food where they remained until 7 days after irradiation, then again transferred to ordinary food and eggs were collected during 2 days. This latter part of the experiment is grouped as series Br. It should be stressed that there was the same supply of spermatozoa in series Br as compared with series Bq.

Series	Time from irradiation to egg laying	Total chromosomes tested	Lethals in paternal chromosomes
Bq	0—8 hours	871	18 = 2,07 %
Br	7—9 days	648	13 = 2,01 »
Total		1519	31 = 2,04 %

There is no difference between the rates in Bq and Br, so we may use the pooled percentage of 2,04. This percentage is in very good agreement with that found by SPENCER and STERN (1948).

The difference between Bf and Bq + Br is $1{,}44 \pm 0{,}73$ % which has a significance level of P = 0,05. The comparison of series Bd and Be showed that the irradiated cytoplasm does not induce mutations in paternal chromosomes if fertilization takes place more than a rather limited time after irradiation. The comparison of Bf *v.* Bq + Br does, however, indicate that when fertilization takes place only a few hours after irradiation there may be an indirect mutagenic influence from the irradiated cytoplasm.

Summary. — Experiments were made on the mutagenic effect on paternal chromosomes of x-raying egg cytoplasm in *Drosophila melanogaster*. The results indicate that if fertilization takes place shortly after irradiation such an effect may be produced. Further experiments are, however, desirable.

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KERSTIN GEZELIUS and NILS FRIES: Phage resistant mutants induced in *Escherichia coli* by caffeine.

Caffeine has earlier been shown to produce mutations in fungi (FRIES and KIHLMAN, 1948) as well as in higher plants (KIHLMAN and LEVAN, 1949; KIHLMAN, 1951). It is also mentioned by DEMEREC, WALLACE and WITKIN (1948) among several other substances capable of inducing mutations to phage-resistance in *Escherichia coli*. However, no quantitative data as to this effect seem to be published. In a recent paper DEMEREC, BERTANI and FLINT (1951) have also demonstrated a rather weak mutagenic activity of caffeine, as tested for the ability of inducing back-mutations from streptomycin dependence to non-dependence in *E. coli*.

Since we were particularly interested in caffeine and other alkyl derivatives of purines as mutagens, we performed a few experiments with *E. coli*, applying the method worked out by DEMEREC (1946), DEMEREC and LATARJET (1946) and WITKIN (1947). The strain of *E. coli* B was obtained through the courtesy of Dr. C.-G. HEDÉN, Stockholm, as well as the T1 strain of the bacteriophage. The method used agreed essentially with that described by WITKIN (1947). Consequently only zero point mutations to phage resistance were determined.

Preliminary experiments showed that *E. coli* was much less sensitive than *Ophiostoma* and higher plants to caffeine and other alkylated purines. Therefore, the highest possible concentration of caffeine, *i. e.* 2 %, had to be used in order to produce a not too slow killing effect. After an exposure of 5 1/2 hours to this solution, 8 % of the cells survived, after 9 hours, 0,4—0,8 %. Four separate experiments were performed, and in all of them a distinct increase in the number of phage-resistant mutants was obtained by the treatment with caffeine (Table 1).

Two reconstruction experiments showed that the death rate in a caffeine solution was about the same for sensitive and resistant cells (Table 2). Thus, a direct selection effect being excluded, it seems permitted to conclude that the increased percentage of mutant cells in the series treated is due to the mutagenic action of caffeine.

In the mutation experiments summarized in Table 1 the number of phage particles was more or less inferior to that of the (dead and living) bacterial cells simultaneously present. Since killed bacteria are still able to absorb phage, the number of phage particles employed may be insufficient completely

TABLE 1. *Mutations to T1 resistance induced by exposure to 2 % caffeine.*

Expt. No.	Treatment (hours)	Survival %	Ratio bacteria to phage	Control		Treated	
				Total No. of bacteria per ml	Mutants per 10^8 bacteria	Total No. of bacteria per ml	Mutants per 10^8 bacteria
1	5 1/2	8,2	2,2	$2{,}2 \cdot 10^{10}$	28,6	$1{,}8 \cdot 10^9$	50,6
2	9	0,8	8,0	$2{,}4 \cdot 10^{10}$	31,8	$2{,}0 \cdot 10^8$	216,9
3	9	0,4	1,4	$2{,}8 \cdot 10^9$	97,1	$1{,}1 \cdot 10^7$	642,9
4	9	0,8	3,1	$6{,}2 \cdot 10^9$	23,9	$5{,}1 \cdot 10^7$	188,1

to prevent phenotypic lag in sensitive bacteria (BEALE, 1947; LEDERBERG, 1949). The relatively long time of exposure to caffeine can also be assumed to increase the danger of false zero mutations appearing on the plates. However, the assumption of such a possible source of error does not agree with the results of the two experiments Nos. 2 and 4, which coincide very well with each other, despite the great difference in the bacteria: phage ratio.

TABLE 2. *Tests for differential killing by exposure to 2 % caffeine.*

Control = mixed culture in distilled water; Treated = mixed culture in 2 % caffeine.

Expt. No.	Control				Treated			
	Survivors		Mutants		Survivors		Mutants	
	No. per ml	% of control	No. per ml	% of survivors	No. per ml	% of treated	No. per ml	% of survivors
5	$9{,}3 \cdot 10^9$	100	$4{,}7 \cdot 10^9$	51,0	$1{,}3 \cdot 10^9$	13,6	$7{,}0 \cdot 10^7$	53,2
6	$6{,}3 \cdot 10^9$	100	$1{,}8 \cdot 10^9$	29,3	$4{,}4 \cdot 10^6$	0,07	$1{,}0 \cdot 10^6$	23,6

It was our intention also to test the activity of other alkylated purines, particularly that of the 8-ethers and 8-thioethers. However, experiments with two derivatives belonging to the most active ones in causing changes in the chromosome structure of onion roots, *viz.* 8-ethoxycaffeine and 8-ethyl-thiocaffeine (KIHLMAN, 1951), failed because of their low solubility and tardy killing effect. Tetrametyluric acid, on the other hand, proved to be more toxic than caffeine in *E. coli*, but for some unknown reason the death rate varied enormously from one experiment to another. Consequently, no reproducible killing curve could so far be established as a basis of mutagenicity tests.

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